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(57) Abstract

The invention relates to modified immunoglobulin molecules in which one or more variable region residues that form intrachain disulfide bonds are substituted with amino acid residues that do not contain sulfydryl groups, such that the intrachain disulfide bond does not form. Such immunoglobulin molecules have an enhanced ability to elicit an anti-idiotype response. The invention further provides for the methods of prevention and treatment of cancer and/or infectious diseases using the modified immunoglobulins of the invention.

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MODIFIED ANTIBODIES WITH ENHANCED ABILITY TO ELICIT AN ANTI-IDIOTYPE RESPONSE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of Provisional application Serial No. 60/065,716, filed November 14, 1997, and Provisional application Serial No. 60/081,403, filed April 10, 1998, both of which are incorporated by reference herein in their entireties.

1. FIELD OF THE INVENTION

The present invention relates to modified immunoglobulins, and vaccine compositions thereof, in which one or more variable region cysteine residues that form intrachain disulfide bonds have been replaced with amino acid residues that do not contain a sulfhydryl group and, therefore, do not form disulfide bonds. The present invention also relates to use of the vaccine compositions of the invention to treat or prevent certain diseases and disorders, particularly cancers and infectious diseases.

2. BACKGROUND OF THE INVENTION

2.1. IMMUNOGLOBULIN STRUCTURE

The basic unit of immunoglobulin structure is a complex of four polypeptides -- two identical low molecular weight or "light" chains and two identical high molecular weight or "heavy" chains, linked together by both noncovalent associations and by disulfide bonds. Each light and heavy chain of an antibody has a variable region at its amino terminus and a constant domain at its carboxyl terminus (Figure 1). The variable regions are distinct for each antibody and contain the antibody antigen binding site. Each variable domain is comprised of four relatively conserved framework regions and three regions of sequence hypervariability termed complementarity determining regions or CDRs (Figure 2). For the most part, it is the CDRs that form the antigen binding site and confer antigen specificity. The constant regions are more highly conserved than the variable domains, with slight variations due to haplotypic differences.

Based on their amino acid sequences, light chains are classified as either kappa or lambda. The constant region heavy chains are composed of multiple domains (CH1, CH2, CH3...CHx), the number depending upon the particular antibody class. The CH1 region is separated from the CH2 region by a hinge region which allows flexibility in the antibody. The variable region of each light chain aligns with the variable region of each heavy chain,

and the constant region of each light chain aligns with the first constant region of each heavy chain. The CH2-CHx domains of the constant region of a heavy chain form an "Fc region" which is responsible for the effector functions of the immunoglobulin molecule, such as complement binding and binding to the Fc receptors expressed by lymphocytes, granulocytes, monocyte lineage cells, killer cells, mast cells and other immune effector cells.

As seen in Figure 3, the light and heavy chains of an IgG molecule form the variable region domain and the constant region domain. Each domain is composed of a sandwich of two parallel extended protein layers of about 100 amino acids in length which are connected by a single disulfide bond (See Roitt et al., Immunology, 3rd Edition, London; Mosby, 1993, p 4.4). Each of the two extended protein layers of the domain, in turn, contains two "anti-parallel" adjacent strands which adopt a beta-sheet conformation. (See, *e.g.*, Stryer, 1975, Biochemistry, WH Freeman and Co., p. 950). Each of the domains has a similar three-dimensional structure based on the immunoglobulin fold.

2.2. IMMUNOTHERAPY AND ANTI-IDIOTYPE ANTIBODIES

In modern medicine, immunotherapy or vaccination has virtually eradicated diseases such as polio, tetanus, tuberculosis, chicken pox, measles, hepatitis, etc. The approach using vaccinations has exploited the ability of the immune system to prevent infectious diseases.

Use of immunotherapy has also been explored for cancer therapy. The era of tumor immunology began with experiments by Prehn and Main, who showed that antigens on the methylcholanthrene (MCA)-induced sarcomas were tumor specific in that transplantation assays could not detect these antigens in normal tissue of the mice (Prehn et al., 1957, J. Natl. Cancer Inst. 18:79-778). This notion was confirmed by further experiments demonstrating that tumor specific resistance against MCA-induced tumors could be elicited in the autochthonous host, that is, the mouse in which the tumor originated (Klein et al., 1990, Cancer Res. 20:151-1572).

There are many reasons why immunotherapy is desired for use in cancer patients. First, if cancer patients are immunosuppressed in surgery, with anesthesia and subsequent chemotherapy, it may worsen the immunosuppression, then with appropriate immunotherapy in the preoperative period, this immunosuppression may be prevented or reversed. This could lead to fewer infectious complications and accelerated wound healing. Second, tumor bulk is minimal following surgery and immunotherapy is most likely to be effective in this situation. A third reason is the possibility that tumor cells are shed into the circulation at surgery and effective immunotherapy applied at this time can eliminate these cells.

There are two types of immunotherapy, the "active immunotherapy" and the "passive immunotherapy". In "active immunotherapy", an antigen is administered in the form of a vaccine, to a patient, so as to elicit a protective immune response. "Passive immunotherapy" involves the administration of antibodies to a patient without eliciting a concommitant immune response. When a specific antibody from one animal is injected as an immunogen into a suitable second animal, the injected antibody will elicit an immune response. Antibody therapy is conventionally characterized as passive since the patient is not the source of the antibodies. However, the term passive is misleading because the patient can produce anti-idiotypic secondary antibodies which in turn provoke an immune response which is cross-reactive with the original antigen. Immunotherapy where the patient generates secondary antibodies is often more therapeutically effective than passive immunotherapy because the patient's own immune system continues to fight the cells bearing the particular antigen well after the initial infusion of antibody.

In an anti-idiotype response, antibodies produced initially during an immune response or introduced into an organism will carry unique new epitopes to which the organism is not tolerant, and therefore will elicit production of secondary antibodies (termed "Ab2"), some of which are directed against the idiotype (*i.e.*, the antigen binding site) of the primary antibody (termed "Ab1"), *i.e.*, the antibody that was initially produced or introduced exogenously. These secondary antibodies or Ab2 likewise will have an idiotype, which will induce production of tertiary antibodies (termed "Ab3"), some of which will recognize the antigen binding site of Ab2, and so forth. This is known as the "network" theory. Some of the secondary antibodies will have a binding site which is an analog of the original antigen, and thus will reproduce the "internal image" of the original antigen. And, the tertiary or Ab3 antibodies that recognize this antigen binding site of the Ab2 antibody will also recognize the original antigen (Figure 4).

Therefore, anti-idiotypic antibodies have binding sites that are similar in conformation and charge to the antigen, and can elicit the same or greater response than that of the cancer antigen itself. Administration of an exogenous antibody that can elicit a strong anti-idiotypic response can thus serve as an effective vaccine, by maintaining a constant immune response.

To date, anti-idiotypic vaccines have comprised murine antibodies because the anti-idiotypic response occurs as part of the typical human anti-mouse antibody (HAMA) response. A strong anti-idiotypic cascade has been observed when Ab1 has been structurally damaged (Madiyalakan et al., 1995, *Hybridoma* 14:199-203), rendering the antibody more foreign. There has been direct administration to the subject of exogenously produced anti-

idiotype antibodies that are raised against the idiotype of an anti-tumor antibody (U.S. Patent No. 4,918,14). After administration, the subject's body will produce anti-antibodies which not only recognize these anti-idiotype antibodies, but also recognize the original tumor epitope, thereby directing complement activation and other immune system responses to a foreign entity to attack the tumor cell that expresses the tumor epitope.

However, while anti-idiotypic vaccines are desirable targets and several have been identified, the ability to deliver antibodies that reproducibly cause the generation of such an anti-idiotypic response is not currently possible. (Foon et al., 1995, *J. Clin. Invest.* 9:334-342; Madiyalakan et al., 1995, *Hybridoma* 14:199-203). One of the reasons for the failure to generate an anti-idiotypic response is that, Ab1, while exogenous, is still very similar to "self", as all antibodies have very similar structures, and anti-idiotypic responses to self molecules tend to be very limited. Thus, there is a need in the art for methods of reliably generating an anti-idiotype response to a specific antibody.

3. SUMMARY OF THE INVENTION

The present invention is based upon the realization of the present inventors that an antibody in which one or more variable region cysteine residues that form one or more intrachain disulfide bonds have been replaced with amino acid residues that do not contain sulfhydryl groups, such that the particular disulfide bonds do not form, elicit a much stronger anti-idiotype response than an antibody in which the variable region disulfide bonds are intact.

Accordingly, the present invention provides modified immunoglobulin molecules or antibodies (and functionally active fragments, derivatives and analogs thereof), and vaccine compositions containing these immunoglobulin molecules, wherein the variable region of the immunoglobulin is subject to decreased conformational constraints, such as, but not limited to, by breaking one or more intrachain or interchain disulfide bonds. Specifically, the invention provides modified immunoglobulins that comprise a variable region and are identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding (*i.e.*, specific binding of the immunoglobulin to its antigen as determined by any method known in the art for determining antibody-antigen binding, which excludes non-specific binding but not necessarily cross-reactivity with other antigens) an antigen, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second

immunoglobulin molecule. In preferred embodiments, the second immunoglobulin molecule can immunospecifically bind a cancer antigen; in other preferred embodiments, the second immunoglobulin molecule can immunospecifically bind an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent.

The invention further provides methods of eliciting an anti-idiotype response in a subject by administering the modified immunoglobulins of the invention. In particular embodiments, the modified immunoglobulins of the invention can be used to treat or prevent cancer, specifically by administering an immunoglobulin molecule of the invention, which immunoglobulin molecule was derived (*i.e.*, by modification according to the invention to replace one or more variable region cysteine residues that form an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group) from an immunoglobulin molecule that can immunospecifically bind a cancer antigen, the expression of which cancer antigen is associated with the particular type of cancer. Additionally, in other embodiments, the modified immunoglobulin molecules of the invention can be used to treat or prevent an infectious disease by administering an immunoglobulin molecule derived from an immunoglobulin molecule that can immunospecifically bind an antigen of or a cellular receptor for the infectious disease agent causing the infectious disease.

The invention also provides methods of producing the modified immunoglobulin molecules of the invention and vaccine compositions containing the modified immunoglobulin molecules of the invention.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1. A schematic diagram showing the structure of the light and heavy chain of an immunoglobulin molecule, each chain consisting of a variable region positioned at the amino terminal region (H_2N-) and a constant region positioned at a carboxyl terminal region (-COOH).

Figure 2. A schematic diagram of an IgG showing the four framework regions (FR1, FR2, FR3 and FR4) and three complementarity determining regions (CDR1, CDR2 and CDR3) in the variable regions of the light and heavy chains (labeled as V_L and V_H, respectively). The constant region domains are indicated as C_L for the light chain constant domain and CH_1, CH_2 and CH_3 for the three domains of the heavy chain constant region. Fab indicates the portion of the antibody fragment which includes the variable region domains of both light and heavy chains and the C_L and CH_1 domains. Fc indicates the constant region fragment containing the CH_2 and CH_3 domains.

Figure 3. A schematic diagram of an antibody structure as shown in Figure 2, but drawn to emphasize that each domain (the loop structures labeled as V_L, V_H, C_L, CH_1, CH_2, and CH_3, respectively) is structurally defined by a disulfide bond (indicated with darkest lines) that maintains the three-dimensional structure (Roitt et al., *Immunology*, Second Edition, London: Gower Medical Publishing, 1989, p 5.3).

Figure 4. A schematic diagram showing the development of internal image bearing anti-idiotype antibodies (Ab2) and anti-anti-idiotype antibodies (Ab3) from idiotype antibodies (Ab1) directed against a ligand in an anti-idiotypic cascade.

Figure 5. Modification of the variable region of an immunoglobulin by replacing cysteine residues in the variable regions with alanine residues to break a variable region intrachain disulfide bond. CH1, CH2 and CH3 are constant regions. V_H is the heavy chain variable region and V_L is the light chain variable region.

Figures 6A-C. (A). The structure of the expression vector pMRRO10.1, which contains a human kappa light chain constant region sequence. (B). The structure of the expression vector pGamma1 that contains a sequence encoding a human IgG1 constant region (CH1, CH2, CH3) heavy chain and hinge region sequences. (C) The structure of the expression vector pNEPuDGV which contains a sequence encoding the kappa constant domain of the light chain and the constant domain and hinge region of the heavy chain. For all three vectors see Bebbington et al., 1991, *Methods in Enzymology* 2:136-145.

Figures 7A and B. (A) The amino acid sequence and corresponding nucleotide sequence including the leader sequence for the consensus light chain variable region ConVL1. (B) The amino acid and corresponding nucleotide sequences for the consensus heavy chain variable region ConVH1 including the leader sequence.

Figures 8A-B. (A) Amino acid and corresponding nucleotide sequence of 2CAVLCOL1, which is the light chain variable region sequences of an antibody derived from mAb31.1, in which alanine residues have been substituted for cysteine residues at positions 23 and 88, which residues are boxed. (B) Amino acid and corresponding nucleotide sequence of 2CAVHCOL1, which is the heavy chain variable region sequence of an antibody derived from mAb31.1, in which alanine residues have been substituted for cysteine residues at positions 23 and 88, which residues are boxed.

Figures 9A-D. (A) Oligonucleotide sequences for the oligonucleotides used to assemble 2CAVHCOL1, the heavy chain variable region gene specific to human colon cancer antigen. (B) Oligonucleotide sequences for the oligonucleotides used to assemble the 2CAVLCOL1 light chain variable region gene specific to human colon cancer antigen. (C) Oligonucleotide sequences for the oligonucleotides used to assemble the light chain

consensus region referred to as ConVL1. (D) Oligonucleotide sequences for the oligonucleotides used to assemble the heavy chain consensus region referred to as ConVL1.

Figure 10. A schematic diagram of the general steps that were followed for the assembly of an engineered gene encoding the synthetic modified antibody specific to human colon cancer antigen.

Figure 11. Dot blot showing the result of an assay for the competition of binding of the antibody derived from mAB31.1, but not having the cysteine to alanine changes with the same antibody which is biotin labeled to an antigen preparation derived from LS-174 T-cells. The concentration of the unlabeled antibody is indicated as nM unlabeled antibody. The "blk" lane has no antigen.

Figures 12A-D. (A)Results of competition binding assay of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody but has not been modified, and peptides CDR1, CDR2, CDR3, CDR4, CDR5, and CDR6, having the CDR sequences containing the bradykinin receptor binding site expressed as percent of control binding to LS-174T cells. (B). Results of competition binding assays of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody, but has not been modified, 2CAVHCOL1, and 2CAVLCOL1. (C) Diagram showing the binding of a biotin-labeled (indicated by the "b") antibody (inverted Y) to antigen (solid triangles). (D) Diagram showing the inhibition of binding of the biotin-labeled (indicated by the "b") antibody (inverted Y) by anti-idiotype antibodies (solid arrows) to antigen (solid triangles).

Figure 13. Nucleotide sequence for the light chain variable region having a CDR containing a binding sequence for HMFG1.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides modified immunoglobulins (particularly antibodies and functionally active fragments, derivatives, and analogs thereof) that elicit a stronger immune response, particularly a stronger anti-idiotypic response, than the corresponding unmodified immunoglobulins. In particular, the modified immunoglobulins of the invention are immunoglobulins that, when unmodified, immunospecifically bind an antigen, and are modified to decrease the conformational constraints on one variable region of the immunoglobulin molecule, preferably, such that at least one of the cysteines that participates in forming an intrachain disulfide bond in the variable region of the

immunoglobulin has been replaced with an amino acid residue that does not have a sulfhydryl group and, therefore, does not form a disulfide bond, thereby decreasing the conformational constraints of at least one of the variable regions of the immunoglobulin (Figure 5). In preferred embodiments of the invention, the modified immunoglobulin molecule is derived from an immunoglobulin molecule that is capable of immunospecifically binding a cancer antigen; in other preferred embodiments, the modified immunoglobulin molecule is derived from an immunoglobulin that is capable of immunospecifically binding an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent.

The invention also provides vaccine compositions containing the modified immunoglobulin molecules of the invention. Additionally, the invention provides methods of generating an anti-idiotype response in a subject by administration of the modified immunoglobulin molecules of the invention.

In specific embodiments, the invention provides methods of treating or preventing cancer by administration of a modified immunoglobulin molecule of the invention which, in its unmodified state, is capable of immunospecifically binding a cancer antigen, the expression of which is associated with the particular cancer. Administration of the modified immunoglobulin elicits an anti-idiotype reaction in the subject, leading to the production, by the subject, of antibodies specific for the cancer antigen. In another specific embodiment, the modified immunoglobulin, in its unmodified state, is capable of binding an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent. Such immunoglobulins can be used to treat or prevent the infectious disease caused by the infectious disease agent.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. MODIFIED ANTIBODIES

The modified immunoglobulins, particularly antibodies, of the invention are immunoglobulins that, at least in the unmodified state, can immunospecifically bind an antigen and have been modified to enhance their ability to elicit an anti-idiotype response. Such immunoglobulins are modified to reduce the conformational constraints on a variable region of the immunoglobulin, *e.g.*, by removing or reducing intrachain or interchain disulfide bonds, chemical modification, or any other method known in the art. Specifically, the invention provides a first immunoglobulin molecule that comprises a variable region and that is identical, except for one or more amino acid substitutions in the variable region, to a

second immunoglobulin molecule, the second immunoglobulin molecule being capable of immunospecifically binding an antigen, the amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. The invention also provides nucleic acids containing a nucleotide sequence encoding a modified immunoglobulin of the invention.

Identifying the cysteine residues that form a disulfide bond in a variable region of a particular antibody can be accomplished by any method known in the art. For example, but not by way of limitation, it is well known in the art that the cysteine residues that form intrachain disulfide bonds are highly conserved among antibody classes and across species. Thus, the cysteine residues that participate in disulfide bond formation can be identified by sequence comparison with other antibody molecules in which it is known which residues form a disulfide bond.

Table 1 provides a list of the positions of disulfide bond forming cysteine residues for a number of antibody molecules.

Table 1 (derived from Kabat et al, 1991, sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland).

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Human	kappa light	I	23,88
Human	kappa light	II	23,88
Human	kappa light	III	23,88
Human	kappa light	IV	23,88
Human	lambda light	I	23,88
Human	lambda light	II	23,88
Human	lambda light	IIII	23,88
Human	lambda light	IV	23,88
Human	lambda light	V	23,88
Human	lambda light	VI	23,88
Mouse	kappa light	I	23,88
Mouse	kappa light	II	23,88
Mouse	kappa light	III	23,88
Mouse	kappa light	IV	23,88
Mouse	kappa light	V	23,88
Mouse	kappa light	VI	23,88
Mouse	kappa light	VII	23,88
Mouse	kappa light	Miscellaneous	23,88

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Mouse	lambda light		23,88
Chimpanzee	lambda light		23,88
Rat	kappa light		23,88
Rat	lambda light		23,88
Rabbit	kappa light		23,88
Rabbit	lambda light		23,88
Dog	kappa light		23,88
Pig	kappa light		23 (88)
Pig	lambda light		23,88
Guinea pig	lambda light		23 (88)
Sheep	lambda light		23,88
Chicken	lambda light		23,88
Turkey	lambda light		23 (88)
Ratfish	lambda light		23 (88)
Shark	kappa light		23,88
Human	heavy	I	22,92
Human	heavy	II	22,92
Human	heavy	III	22,92
Mouse	heavy	I (A)	22,92
Mouse	heavy	I (B)	22,92
Mouse	heavy	II (A)	22,92
Mouse	heavy	II (B)	22,92
Mouse	heavy	II (C)	22,92
Mouse	heavy	III (A)	22,92
Mouse	heavy	III(B)	22,92
Mouse	heavy	III (C)	22,92
Mouse	heavy	III (D)	22,92
Mouse	heavy	V (A)	22,92
Mouse	heavy	V (B)	22,92
Mouse	heavy	Miscellaneous	22,92
Rat	heavy		22,92
Rabbit	heavy		22,92
Guinea pig	heavy		22,92
Cat	heavy		22 (92)
Dog	heavy		22,92
Pig	heavy		22 (92)
Mink	heavy		22 (92)
Sea lion	heavy		22 (92)
Seal	heavy		22 (92)
Chicken	heavy		22,92
Duck	heavy		22 (92)
Goose	heavy		22 (92)
Pigeon	heavy		22 (92)
Turkey	heavy		22 (92)

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Caiman	heavy		22, 92
Xenopus frog	heavy		22,92
Elops	heavy		22,92
Goldfish	heavy		22,92
Ratfish	heavy		22 (92)
Shark	heavy		22,92

Position numbers enclosed by () indicate that the protein was not sequenced to that position, but the residue is inferred by comparison to known sequences.

Notably, for all of the antibody molecules listed in Table 1, the cysteine residues that form the intrachain disulfide bonds are the residues at positions 23 and 88 of the light chain variable domain and the residues at positions 22 and 92 of the heavy chain variable domain. The position numbers refer to the residue corresponding to that residue in the consensus sequences as defined in Kabat, (1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) or as indicated in the heavy and light chain variable region sequences depicted in Figures 7A and B, respectively ("corresponding" means as determined by aligning the particular antibody sequence with the consensus sequence or the heavy or light chain variable region sequence depicted in Figure 7A or B).

Accordingly, in one embodiment of the invention, the modified immunoglobulin molecule is an antibody in which the residues at positions 23 and/or 88 of the light chain are substituted with an amino acid residue that does not contain a sulfhydryl group and/or the residues at positions 22 and/or 92 of the heavy chain are substituted with an amino acid residue that does not contain a sulfhydryl group.

In the modified immunoglobulin of the invention, the amino acid residue that substitutes for the disulfide bond forming cysteine residue is any amino acid residue that does not contain a sulfhydryl group, *e.g.*, alanine, arginine, asparagine, aspartate (or aspartic acid), glutamine, glutamate (or glutamic acid), glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. In a preferred embodiment, the cysteine residue is replaced with a glycine, serine, threonine, tyrosine, asparagine, or glutamine residue, most preferably, with an alanine residue.

Additionally, the disulfide bond forming cysteine residue may be replaced by a nonclassical amino acid or chemical amino acid analog that does not contain a sulfhydryl

group (for example, but not by way of limitation, using routine protein synthesis methods). Non-classical amino acids include, but are not limited, to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, γ-Abu, ϵ-Ahx, -amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary). In an alternative embodiment, the disulfide bond forming residue is deleted.

In specific embodiments, the substitution of the disulfide bond forming residue is in the heavy chain variable region or is in the light chain variable region or is in both the heavy chain and light chain variable regions. In other specific embodiments, one of the residues that forms a particular disulfide bond is replaced (or deleted) or, alternatively, both residues that form a particular disulfide bond may be replaced (or deleted).

In other embodiments, the invention provides immunoglobulin molecules that have one or more amino acid substitutions relative to the second immunoglobulin molecule of a disulfide bond forming residue in the variable region with an amino acid residue that does not contain a sulfhydryl group and that additionally have one or more other amino acid substitutions (*i.e.*, that are not a replacement of a disulfide bond forming residue with a residue that does not contain a sulfhydryl group).

In particular, the invention provides a first immunoglobulin molecule comprising a variable region and which is identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, in which at least one of said one or more amino acid substitutions are the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

In a preferred embodiment, the amino acid substitutions that are not the substitution of a disulfide bond forming cysteine residue with a residue that does not have a sulfhydryl group, are not stabilizing changes. Stabilizing changes are defined as those amino acid changes that increase the stability of the antibody molecule. Such stabilizing amino acid changes are those changes that substitute an amino acid that is not common at that particular position in the particular antibody molecule (*e.g.*, as defined by the consensus sequences for a number of antibody molecules provided in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda,

Maryland) with a residue that is common at that particular position, *e.g.*, is the amino acid at that position in the consensus sequence for that antibody molecule (see PCT Publication WO 96/02574, dated February 1, 1996 by Steipe et al.).

Such other amino acid substitutions can be any amino acid substitution that does not alter the ability of the modified immunoglobulin to elicit the formation of anti-anti-idiotype antibodies, *e.g.*, as determined, for example, as described in Section 5.5, *infra*. For example, such other amino acid substitutions include substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues can be substituted by another amino acid of a similar polarity which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The modified antibodies of the invention can be derived from antibodies that are capable of immunospecifically binding any antigen. In a preferred embodiment, the modified antibodies are derived from antibodies that are capable of immunospecifically binding a cancer antigen, more preferably a tumor antigen. In specific embodiments, the modified antibodies are derived from antibodies that are capable of binding polymorphic epithelial mucin antigen, human colon carcinoma-associated protein antigen, human colon carcinoma-associated carbohydrate antigen, human milk fat globule, or is an antigen of a cancer of the breast, ovary, uterus, prostate, bladder, lung, skin, colon, pancreas, gastrointestinal track, B lymphocytes or T lymphocytes or any other cancer characterized by the expression of specific antigens, *e.g.*, those discussed in Section 5.2.1, *infra*. In preferred embodiments, the modified antibody is derived from Mab 31.1 (available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2201 under No. 12314), Mab 33.28 (under No. 12315) or Mab HMFG-1 (see PCT Publication WO90/05142 and PCT Publication WO92/04380).

In another specific embodiment, the modified antibodies of the invention are derived from antibodies that are capable of immunospecifically binding an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent. In preferred embodiments, the antigen of the infectious disease agent is a bacterial antigen, a viral

antigen, or an antigen of a parasite, or any other antigen of an infectious disease agent, such as those infectious disease agents described in Section 5.2.2, *infra*.

The immunoglobulin molecules of the invention can be of any type, class, or subclass of immunoglobulin molecules. In a preferred embodiment, the immunoglobulin molecule is an antibody molecule, more preferably of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA, most preferably is an IgG molecule. Alternatively, the immunoglobulin molecule is a T cell receptor, a B cell receptor, a cell-surface adhesion molecule such as the co-receptors CD4, CD8, or CD19, or an invariant domain of an MHC molecule.

The modified immunoglobulin can be derived from any naturally occurring antibody, preferably a monoclonal antibody, or can be derived from a synthetic or engineered antibody. In one aspect of the invention, the modified immunoglobulin molecules are derived from an antibody in which a binding site for a member of a binding pair or a portion of an antigen is inserted into or replaces all or a portion of one of the CDRs in the variable region, for example as described in co-pending United States Patent application Serial No.___, entitled "Immunoglobulin Molecules Having A Synthetic Variable Region And Modified Specificity", by Burch, filed November 13, 1998 (attorney docket no. 6750-016), which is incorporated by reference herein in its entirety.

In particular, the synthetic antibodies are antibodies that immunospecifically bind to a first member of a binding pair where at least one of the CDRs of the antibody contains a binding site for the first member of the binding pair, which binding site is derived from an amino acid sequence of the other member of the binding pair. In one aspect of the invention, the amino acid sequence of the binding site is not found naturally within the CDR. Additionally, at least one of the CDRs may contain a portion of an antigen, particularly an epitope.

The amino acid sequence of the binding site may be identified by any method known in the art. For example, in some instances, the sequence of a member of a binding pair has already been determined to be directly involved in binding the other member of the binding pair. In this case, such a sequence can be used to construct the CDR of a synthetic antibody that specifically recognizes the other member of the binding pair. If the amino acid sequence for the binding site in the one member of the binding pair for the other member of the binding pair is not known, it can be determined by any method known in the art, for example, but not limited to, molecular modeling methods or empirical methods, *e.g.*, by assaying portions (*e.g.*, peptides) of the member for binding to the other member, or by making mutations in the member and determining which mutations prevent binding.

The binding pair can be any two molecules, including proteins, nucleic acids, carbohydrates, or lipids, that interact with each other, although preferably the binding partner from which the binding site is derived is a protein molecule. In preferred embodiments, the modified immunoglobulin contains a binding sequence for a cancer antigen, an infectious disease antigen, a cellular receptor for a pathogen, or a receptor or ligand that participates in a receptor-ligand binding pair.

In specific embodiments, the binding pair is a protein-protein interaction pair which is either homotypic interaction (*i.e.*, is the interaction between two of the same proteins) or a heterotypic interaction (*i.e.*, is the interaction between two different proteins).

In a specific embodiment, the first member is a member of a ligand-receptor binding pair, preferably, of a receptor-ligand binding pair in which the ligand binds to the receptor and thereby elicits a physiological response, such as intracellular signaling. By way of example, and not by way of limitation, the ligand or receptor can be a hormone, autocoid, growth factor, cytokine or neurotransmitter, or receptor for a hormone, autocoid, growth factor, cytokine, or neurotransmitter, or any receptor or ligand involved in signal transduction. (For reviews of signal transduction pathways, see, *e.g.*, Campbell, 1997, *J. Pediat.* 131:S42-S44; Hamilton, 1997, *J. Leukoc. Biol.* 62:145-155; Soede-Bobok & Touw, 1997, *J. Mol. Med.* 75:470-477; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., 1994, *Cell* 76:253-262; Miyajima et al., 1992, *Annu. Rev. Immunol.* 10:295-331; and Cantley et al., 1991, *Cell* 64:281-302.). In specific embodiments, one member of the binding pair is ligand such as, but not limited to, cholecystokinin, galanin, IL-1, IL-2, IL-4, IL-5, IL-6, IL-11, a chemokine, leptin, a protease, neuropeptide Y, neurokinin-1, neurokinin-2, neurokinin-3, bombesin, gastrin, corticotropin releasing hormone, endothelin, melatonin, somatostatin, vasoactive intestinal peptide, epidermal growth factor, tumor necrosis factor, dopamine, endothelin, or a receptor for any of these ligands. In other embodiments, one member of the binding pair is a receptor, such as, but not limited to, an opioid receptor, a glucose transporter, a glutamate receptor, an orphanin receptor, erythropoietin receptor, insulin receptor, tyrosine kinase (TK)-receptor, KIT stem cell factor receptor, nerve growth factor receptor, insulin-like growth factor receptor, granulocyte-colony stimulating factor receptor, somatotropin receptor, glial-derived neurotrophic factor receptor or gp39 receptor, G-protein receptor class or ß2-adrenergic receptor, or a ligand that binds any of these receptors. In another embodiment, one of the members of the binding pair is a ligand gated ion channel, such as but not limited to a calcium channel, a sodium channel, or a potassium channel. In certain embodiments, the invention provides modified immunoglobulins that immunospecifically bind a receptor and are antagonists the ligand that binds that receptor,

for example, but not by way of limitation, are antagonists of endorphin, enkephalin or nociceptin. In other embodiments, the invention provides synthetic modified antibodies that immunospecifically bind a receptor and are agonists of the receptor, for example, but not by way of limitation, the endorphin, enkephalin, or nociceptin receptors. In a preferred embodiment, the modified immunoglobulin does not bind the fibronectin receptor. In another preferred embodiment, the binding sequence is not Arg-Gly-Asp, is not a multimer of a binding sequence, and preferably is not a multimer of the sequence Arg-Gly-Asp.

In other specific embodiments, the modified immunoglobulin has a CDR that contains a binding site for a transcription factor. In a preferred aspect, the modified immunoglobulin does not bind to a specific DNA sequence, particularly does not bind to a transcription factor binding site.

In preferred embodiments, the modified immunoglobulin has at least one CDR that contains an amino acid sequence of a binding site for a cancer antigen or a tumor antigen (*e.g.*, as described in detail in section 5.2.1, *infra.*), more preferably the antigen is human colon carcinoma-associated antigen or epithelial mucin antigen. In other embodiments, at least one CDR of the modified immunoglobulin contains an amino acid sequence for a binding site for a human milk fat globule receptor. In other embodiments, the modified immunoglobulin has at least one CDR that contains an amino acid sequence of a binding site for an antigen of a tumor of the breast, ovary, uterus, prostate, bladder, lung, skin, pancreas, colon, gastrointestinal tract, B lymphocytes, or T lymphocytes.

In other preferred embodiments of the invention, at least one CDR of the modified antibody contains an amino acid sequence for a binding site for an antigen of an infectious disease agent (*e.g.*, as described in detail in section 5.2.2, *infra.*), or a binding site for a cellular receptor of an infectious disease agent, preferably where the binding site is not an amino acid sequence of a *Plasmodium* antigen, or is not the binding site Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro. In additional embodiments, the modified antibody has a CDR that contains the binding site for a bacterial or viral enzyme.

The synthetic antibody may be built upon (*i.e.*, the binding site sequences inserted into the CDR of) the sequence of a naturally occurring or previously existing antibody or may be synthesized from known antibody consensus sequences, such as the consensus sequences for the light and heavy chain variable regions in Figures 7A and B, or any other antibody consensus or germline (*i.e.*, unrecombined genomic sequences) sequences (*e.g.*, those antibody consensus and germline sequences described in Kabat et al., 1991, Sequences

of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172).

Each antibody molecule has six CDR sequences, three on the light chain and three on the heavy chain, and five of these CDRs are germline CDRs (*i.e.*, are directly derived from the germline genomic sequence of the animal, without any recombination) and one of the CDRs is a non-germline CDR (*i.e.*, differs in sequence from the germline genomic sequence of the animal and is generated by recombination of the germline sequences). Whether a CDR is a germline or non-germline sequence can be determined by sequencing the CDR and then comparing the sequence with known germline sequences, *e.g.*, as listed in Kabat et al. (1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172). Significant variation from the known germline sequences indicates that the CDR is a non-germline CDR. Accordingly, the CDR that contains the amino acid sequence of the binding site or antigen is a germline CDR or, alternatively, is a non-germline CDR.

The binding site or antigen sequence can be inserted into any of the CDRs of the antibody, and it is within the skill in the art to insert the binding site into different CDRs of the antibody and then screen the resulting modified antibodies for the ability to bind to the particular member of the binding pair, *e.g.* as discussed in Section 5.5, *infra*, or to elicit an immune response against the antigenic site, *e.g.*, as described in Section 5.5, *infra*. Thus, one can determine which CDR optimally contains the binding site or antigen. In specific embodiments, a CDR of either the heavy or light chain variable region is modified to contain the amino acid sequence of the binding site or antigen. In another specific embodiment, the modified antibody contains a variable domain in which the first, second or third CDR of the heavy variable region or the first, second or third CDR of the light chain variable region contains the amino acid sequence of the binding site or antigen. In another embodiment of the invention, more than one CDR contains the amino acid sequence of the binding site or antigen or more than one CDR each contains a different binding site for the same molecule or contains a different binding site for a different molecule. In particular, embodiments, two, three, four, five or six CDRs have been engineered to contain a binding site for the first member of the binding pair. In a preferred embodiment, one or more CDRs contain a binding site for the first member of a binding pair and one or more other CDRs contain a binding site for a molecule on the surface of an immune cell, such as, but not limited to, a T cell, B cell, NK cell, K cell, TIL cell or neutrophil. For example, a modified antibody having a binding site for a cancer antigen or an infectious disease antigen and a

binding site for a molecule on the surface of an immune cell can be used to target the immune cell to a cancer cell bearing the cancer antigen or to the infectious disease agent.

In specific embodiments of the invention, the binding site or antigen amino acid sequence is either inserted into the CDR without replacing any of the amino acid sequence of the CDR itself or, alternatively, the binding site or antigen amino acid sequence replaces all or a portion of the amino acid sequence of the CDR. In specific embodiments, the binding site amino acid sequence replaces 1, 2, 5, 8, 10, 15, or 20 amino acids of the CDR sequence.

The amino acid sequence of the binding site or antigen present in the CDR can be the minimal binding site necessary for the binding of the member of the binding pair or for eliciting an immune response against the antigen(which can be determined empirically by any method known in the art); alternatively, the sequence can be greater than the minimal binding site or antigen sequence necessary for the binding of the member of the binding pair or eliciting of an immune response against the antigen. In particular embodiments, the binding site or antigen amino acid sequence is at least 4 amino acids in length, or is at least 6, 8, 10, 15, or 20 amino acids in length. In other embodiments the binding site amino acid sequence is no more than 10, 15, 20, or 25 amino acids in length, or is 5-10, 5-15, 5-20, 10-15, 10-20 or 10-25 amino acids in length.

In addition, the total length of the CDR (*i.e.*, the combined length of the binding site sequence and the rest of the CDR sequence) should be of an appropriate number of amino acids to allow binding of the antibody to the antigen. CDRs have been observed to have a range of numbers of amino acid residues, and the observed size ranges for the CDRs (as denoted by the abbreviations indicated in figure 2) are provided in Table 2.

Table 2

CDR	Number of residues
L1	10-17
L2	7
L3	7-11
H1	5-7
H2	9-12
H3	2-25

(compiled from data in Kabat and Wu, 1971, *Ann. NY Acad. Sci.* 190:382-93)

While many CDR H3 regions are of 5-9 residue in length, certain CDR H3 regions have been observed that are much longer. In particular, a number of antiviral antibodies have heavy chain CDR H3 regions of 17-24 residues in length.

Accordingly, in specific embodiments of the invention, the CDR containing the binding site or antigen portion is within the size range provided for that particular CDR in Table 2, *i.e.*, if it is the first CDR of the light chain, L1, the CDR is 10 to 17 amino acid residues; if it is the second CDR of the light chain, L2, the CDR is 7 amino acid residues; if it is the third CDR of the light chain, L3, the CDR is 7 to 11 amino acid residues; if it is the first CDR of the heavy chain, H1, the CDR is 5 to 7 amino acid residues; if it is the second CDR of the heavy chain, H2, the CDR is 9 to 12 amino acid residues; and if it is the third CDR of the heavy chain, H3, the CDR is 2 to 25 amino acid residues. In other specific embodiments, the CDR containing the binding site is 5-10, 5-15, 5-20, 11-15, 11-20, 11-25, or 16-25 amino acids in length. In other embodiments, the CDR containing the binding site is at least 5, 10, 15, or 20 amino acids or is no more than 10, 15, 20, 25, or 30 amino acids in length.

After constructing antibodies containing modified CDRs, the modified antibodies can be further altered and screened to select an antibody having higher affinity or specificity. Antibodies having higher affinity or specificity for the target antigen may be generated and selected by any method known in the art. For example, but not by way of limitation, the nucleic acid encoding the synthetic modified antibody can be mutagenized, either randomly, *i.e.*, by chemical or site-directed mutagenesis, or by making particular mutations at specific positions in the nucleic acid encoding the modified antibody, and then screening the antibodies exposed from the mutated nucleic acid molecules for binding affinity for the target antigen. Screening can be accomplished by testing the expressed antibody molecules individually or by screening a library of the mutated sequences, *e.g.*, by phage display techniques (see, *e.g.*, U. S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698, all by Ladner et al; PCT Publication WO 92/01047 by McCafferty et al. or any other phage display technique known in the art).

In specific embodiments, the invention provides a functionally active fragment, derivative or analog of the modified immunoglobulin molecules of the invention. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotype antibodies (*i.e.*, tertiary antibodies or Ab3 antibodies) that recognize the same antigen that the antibody from which the fragment, derivative or analog is derived recognized (*e.g.*, as determined by the methods described in Section 5.5, *infra*). Specifically, in a preferred embodiment, the antigenicity of the idiotype of the immunoglobulin molecule

may be enhanced by deletion of framework and CDR sequences that are N-terminal to the particular CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art. Accordingly, in a preferred embodiment, the invention includes modified immunoglobulin molecules that have one disulfide bond forming cysteine residue in a variable region domain replaced with an amino acid residue that does not contain a sulfhydryl group and in which a portion of that variable domain has been deleted N-terminal to the CDR sequence that recognizes the antigen.

Other embodiments of the invention include fragments of the modified antibodies of the invention such as, but not limited to, $F(ab')_2$ fragments, which contain the variable region, the light chain constant region and the CH1 domain of the heavy chain can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. The invention also provides heavy chain and light chain dimers of the modified antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the modified antibody of the invention.

Techniques have been developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constant domain from a human immunoglobulin, *e.g.*, humanized antibodies.

In a preferred embodiment, the modified immunoglobulin of the invention is a humanized antibody, more preferably an antibody having a variable domain in which the framework regions are from a human antibody and the CDRs are from an antibody of a non-human animal, preferably a mouse (see, International Patent Application No. PCT/GB8500392 by Neuberger et al. and Celltech Limited).

CDR grafting is another method of humanizing antibodies. It involves reshaping murine antibodies in order to transfer full antigen specificity and binding affinity to a human framework (Winter et al. U.S. Patent No. 5,225,539). CDR-grafted antibodies have been

successfully constructed against various antigens, for example, antibodies against IL-2 receptor as described in Queen et al., 1989 (Proc. Natl. Acad. Sci. USA 86:10029); antibodies against cell surface receptors-CAMPATH as described in Riechmann et al. (1988, Nature, 332:323); antibodies against hepatitis B in Cole et al. (1991, Proc. Natl. Acad. Sci. USA 88:2869); as well as against viral antigens-respiratory syncitial virus in Tempest et al. (1991, Bio-Technology 9:267). CDR-grafted antibodies are generated in which the CDRs of the murine monoclonal antibody are grafted into a human antibody. Following grafting, most antibodies benefit from additional amino acid changes in the framework region to maintain affinity, presumably because framework residues are necessary to maintain CDR conformation, and some framework residues have been demonstrated to be part of the antigen binding site. However, in order to preserve the framework region so as not to introduce any antigenic site, the sequence is compared with established germline sequences followed by computer modeling.

In other embodiments, the invention provides fusion proteins of the modified immunoglobulins of the invention (or functionally active fragments thereof), for example in which the modified immunoglobulin is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably an at least 10, 20 or 50 amino acid portion of the protein) that is not the modified immunoglobulin. Preferably the modified immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. In preferred embodiments, the invention provides fusion proteins in which the modified immunoglobulin is covalently linked to IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, γ-interferon, MHC derived peptide, G-CSF, TNF, porins, NK cell antigens, or cellular endocytosis receptor.

The modified immunoglobulins of the invention include analogs and derivatives that are either modified, *i.e*, by the covalent attachment of any type of molecule as long as such covalent attachment does not prevent the modified immunoglobulin from generating an anti-idiotypic response (*e.g.*, as determined by any of the methods described in Section 5.5, *infra*). For example, but not by way of limitation, the derivatives and analogs of the modified immunoglobulins include those that have been further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic

synthesis of tunicamycin, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids, *e.g.*, as listed above in this Section.

Methods of producing the modified immunoglobulins, and fragments, analogs, and derivatives thereof, are described in Section 5.4, *infra*.

5.2. THERAPEUTIC UTILITY

The present invention provides methods of eliciting production of anti-idiotype antibodies and anti-anti-idiotype antibodies in a subject by the administration of a therapeutic (termed herein "Therapeutic"). Such Therapeutics include the modified immunoglobulins of the invention, and functionally active fragments, analogs, and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*), and nucleic acids encoding the modified antibodies of the invention, and functionally active fragments and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*).

Generally, administration of products of a species origin or species reactivity that is the same species as that of the subject is preferred. Thus, in a preferred embodiment, the methods of the invention use a modified antibody that is derived from a human antibody; in other embodiments, the methods of the invention use a modified antibody that is derived from a chimeric or humanized antibody.

Specifically, vaccine compositions (*e.g.*, as described in Section 5.3, *infra*) containing the modified antibodies of the invention are administered to the subject to elicit the production of an antibody (*i.e.*, the anti-idiotype antibody or Ab2) that specifically recognizes the idiotype of the modified antibody, the Ab2, in turn, elicits the production anti-anti-idiotype antibodies (Ab3) that specifically recognize the idiotype of Ab2, such that these Ab3 antibodies have the same or similar binding specificity as the modified antibody.

The invention provides methods of administering the modified antibodies of the invention to elicit an anti-idiotype response, *i.e.*, to generate Ab2 and Ab3 type antibodies. Alternatively, the invention provides methods of administering the modified antibodies of the invention to one subject to generate Ab2 antibodies, isolating the Ab2 antibodies, and then administering the Ab2 antibodies to a second subject to generate Ab3 type antibodies in that second subject.

Accordingly, the invention provides a method of generating an anti-idiotype response in a subject comprising administering an amount of first immunoglobulin molecule (or functionally active fragment, analog, or derivative thereof) sufficient to induce an anti-idiotype response, said first immunoglobulin comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second

immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. In another embodiment, the method further provides isolating the anti-idiotype antibody that recognizes the idiotype of said second immunoglobulin molecule, and administering to a second subject the anti-idiotype antibody.

In particular embodiments discussed in more detail in the subsections that follow, the modified antibodies of the invention may be used to induce an anti-idiotype response to infectious agents and diseased or abnormal cells, such as but not limited to, bacteria, parasites, fungi, viruses, tumors and cancers. The modified antibodies of the invention may be used to either treat or prevent any disease or disorder amenable to treatment or prevention by generating an anit-anti-idiotypic response to a particular antigen.

In other embodiments, the modified antibodies may be used for the treatment of autoimmune disease, such as, but not limited to rheumatoid arthritis, lupus, ulcerative colitis, or psoriasis, or for the treatment of allergies. The methods and vaccine compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against a modified immunoglobulin in a subject. In one specific embodiment, the methods and compositions of the invention elicit a humoral response in a subject. In another specific embodiment, the methods and compositions of the invention elicit a cell-mediated response in a subject. In a preferred embodiment, the methods and compositions of the invention elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice, rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human. The compositions and methods of the invention can be used either to prevent a disease or disorder, or to treat a particular disease or disorder, where an anti-idiotypic response against a particular immunoglobulin molecule is effective to treat or prevent the disease or disorder.

5.2.1. TREATMENT AND PREVENTION OF CANCERS

Cancers, including, but not limited to, neoplasms, tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of a modified immunoglobulin (or functionally active fragment, derivative or analog thereof) of the invention, or a nucleic acid encoding the modified immunoglobulin, or

functionally active fragment, derivative or analog thereof), which modified immunoglobulin is derived from an immunoglobulin that specifically recognizes one or more antigens associated with the cancer cells of the cancer to be treated or prevented. Whether a particular Therapeutic is effective to treat or prevent a certain type of cancer can be determined by any method known in the art, for example but not limited to, those methods described in Section 5.5, *infra*.

For example, but not by way of limitation, cancers associated with the following cancer antigens may be treated or prevented by administration of a modified antibody of the invention derived from an antibody that recognizes these cancer antigens: KS 1/4 pan-carcinoma antigen (Perez and Walker, 1990, *J. Immunol.* 142:32-37; Bumal, 1988, *Hybridoma* 7(4):407-415), ovarian carcinoma antigen (CA125) (Yu et al., 1991, *Cancer Res.* 51(2):48-475), prostatic acid phosphate (Tailor et al., 1990, *Nucl. Acids Res.* 18(1):4928), prostate specific antigen (Henttu and Vihko, 1989, *Biochem. Biophys. Res. Comm.* 10(2):903-910; Israeli et al., 1993, *Cancer Res.* 53:227-230), melanoma-associated antigen p97 (Estin et al., 1989, *J. Natl. Cancer Instit.* 81(6):445-44), melanoma antigen gp75 (Vijayasardahl et al., 1990, *J. Exp. Med.* 171(4):1375-1380), high molecular weight melanoma antigen (HMW-MAA) (Natali et al., 1987, *Cancer* 59:55-3; Mittelman et al., 1990, *J. Clin. Invest.* 86:2136-2144)), prostate specific membrane antigen, carcinoembryonic antigen (CEA) (Foon et al., 1994, *Proc. Am. Soc. Clin. Oncol.* 13:294), polymorphic epithelial mucin antigen, human milk fat globule antigen, Colorectal tumor-associated antigens such as: CEA, TAG-72 (Yokata et al., 1992, *Cancer Res.* 52:3402-3408), CO17-1A (Ragnhammar et al., 1993, *Int. J. Cancer* 53:751-758); GICA 19-9 (Herlyn et al., 1982, *J. Clin. Immunol.* 2:135), CTA-1 and LEA, Burkitt's lymphoma antigen-38.13, CD19 (Ghetie et al., 1994, *Blood* 83:1329-1336), human B-lymphoma antigen-CD20 (Reff et al., 1994, *Blood* 83:435-445), CD33 (Sgoûros et al., 1993, *J. Nucl. Med.* 34:422-430), melanoma specific antigens such as ganglioside GD2 (Saleh et al., 1993, J.Immunol., 151, 3390-3398), ganglioside GD3 (Shitara et al., 1993, *Cancer Immunol. Immunother.* 36:373-380), ganglioside GM2 (Livingston et al., 1994, *J. Clin. Oncol.* 12:1036-1044), ganglioside GM3 (Hoon et al., 1993, *Cancer Res.* 53:5244-5250), tumor-specific transplantation type of cell-surface antigen (TSTA) such as virally-induced tumor antigens including T-antigen DNA tumor viruses and envelope antigens of RNA tumor viruses, oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (Hellstrom et al., 1985, *Cancer. Res.* 45:2210-2188), differentiation antigen such as human lung carcinoma antigen L6, L20 (Hellstrom et al., 1986, *Cancer Res.* 46:3917-3923), antigens of fibrosarcoma, human leukemia T cell antigen-Gp37 (Bhattacharya-Chatterjee et al., 1988, *J. of Immun.*

141:1398-1403), neoglycoprotein, sphingolipids, breast cancer antigen such as EGFR (Epidermal growth factor receptor), HER2 antigen ($p185^{HER2}$), polymorphic epithelial mucin (PEM) (Hilkens et al., 1992, *Trends in Bio. Chem. Sci.* 17:359), malignant human lymphocyte antigen-APO-1 (Bernhard et al., 1989, *Science* 245:301-304), differentiation antigen (Feizi, 1985, *Nature* 314:53-57) such as I antigen found in fetal erthrocytes and primary endoderm, I(Ma) found in gastric adencarcinomas, M18 and M39 found in breast epithelium, SSEA-1 found in myeloid cells, VEP8, VEP9, Myl, VIM-D5,and $D_1$56-22 found in colorectal cancer, TRA-1-85 (blood group H), C14 found in colonic adenocarcinoma, F3 found in lung adenocarcinoma, AH6 found in gastric cancer, Y hapten, Le^y found in embryonal carcinoma cells, TL5 (blood group A), EGF receptor found in A431 cells , E_1 series (blood group B) found in pancreatic cancer, FC10.2 found in embryonal carcinoma cells, gastric adenocarcinoma, CO-514 (blood group Le^a) found in adenocarcinoma, NS-10 found in adenocarcinomas, CO-43 (blood group Le^b), G49, EGF receptor, (blood group ALe^b/Le^y) found in colonic adenocarcinoma, 19.9 found in colon cancer, gastric cancer mucins, T_5A_7 found in myeloid cells, R_{24} found in melanoma, 4.2, G_{D3}, D1.1, OFA-1, G_{M2}, OFA-2, G_{D2}, M1:22:25:8 found in embryonal carcinoma cells and SSEA-3, SSEA-4 found in 4-8-cell stage embryos. In another embodiment, the antigen is a T cell receptor derived peptide from a cutaneous T cell lymphoma (see Edelson, 1998, *The Cancer Journal* 4:62).

In other embodiments of the invention, the subject being treated with the modified antibody of this invention may, optionally, be treated with other cancer treatments such as surgery, radiation therapy or chemotherapy. In particular, the Therapeutic of the invention used to treat or prevent cancer may be administered in conjunction with one or a combination of chemotherapeutic agents including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, an etoposide, a campathecin, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc.

5.2.1.1. MALIGNANCIES

Malignancies and related disorders that can be treated or prevented by administration of the invention include but are not limited to those listed in Table 3 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 3
MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma
    - chondrosarcoma
    - osteogenic sarcoma
    - chordoma
    - angiosarcoma
    - endotheliosarcoma
    - lymphangiosarcoma
    - lymphangioendotheliosarcoma
    - synovioma
    - mesothelioma
    - Ewing's tumor
    - leiomyosarcoma
    - rhabdomyosarcoma
    - colon carcinoma
    - pancreatic cancer
    - breast cancer
    - ovarian cancer
    - prostate cancer
    - squamous cell carcinoma
    - basal cell carcinoma
    - adenocarcinoma
    - sweat gland carcinoma
    - sebaceous gland carcinoma
    - papillary carcinoma
    - papillary adenocarcinomas

	cystadenocarcinoma
	medullary carcinoma
	bronchogenic carcinoma
	renal cell carcinoma
	hepatoma
	bile duct carcinoma
5	choriocarcinoma
	seminoma
	embryonal carcinoma
	Wilms' tumor
	cervical cancer
	uterine cancer
	testicular tumor
10	lung carcinoma
	small cell lung carcinoma
	bladder carcinoma
	epithelial carcinoma
	glioma
	astrocytoma
	medulloblastoma
	craniopharyngioma
15	ependymoma
	pinealoma
	hemangioblastoma
	acoustic neuroma
	oligodendroglioma
	meningioma
	melanoma
	neuroblastoma
20	retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the 25 ovary, bladder, breast, colon, lung, skin, pancreas, or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented.

5.2.1.2. PREMALIGNANT CONDITIONS

The Therapeutics of the invention can also be administered to treat premalignant 30 conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 3. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see 35 Robbins and Angell, 197, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp.

8-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of the vaccine composition. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of the Therapeutic of the invention: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease

showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 197, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, Therapeutic of the invention is administered to a human patient to prevent progression to ovary, breast, colon, lung, pancreatic, skin, prostate, gastrointestinal, B lymphocyte, T lymphocyte or uterine cancer, melanoma or sarcoma.

5.2.2. TREATMENT OF INFECTIOUS DISEASES

The invention also provides methods of treating or preventing infectious diseases by administration of a Therapeutic of the invention, in particular a modified immunoglobulin molecule (or functionally active fragment, derivative or analog thereof, or a nucleic acid encoding the modified immunoglobulin, or functionally active fragment, analog or derivative thereof) that is derived from an immunoglobulin molecule that can immunospecifically bind an antigen of the agent causing the infectious disease or a cellular receptor for the infectious disease agent. As discussed in detail below, the infectious agents include, but are not limited to viruses, bacteria, fungi, protozoa, and parasites.

In specific embodiments, infectious diseases are treated or prevented by administration of a modified immunoglobulin of the invention (or functionally active fragment, derivative or analog thereof, or nucleic acid encoding the same) that is derived from an immunoglobulin that specifically recognizes one of the following antigens of an infectious disease agent: influenza virus hemagglutinin (Genbank accession no. JO2132; Air, 1981, *Proc. Natl. Acad. Sci. USA* 78:739-743; Newton et al., 1983, *Virology* 128:495-501), human respiratory syncytial virus G glycoprotein (Genbank accession no. Z33429; Garcia et al., 1994, *J. Virol.*; Collins et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:783), core protein, matrix protein or other protein of Dengue virus (Genbank accession no. M19197; Hahn et al., 1988, *Virology* 12:17-180), measles virus hemagglutinin (Genbank accession no. M81899; Rota et al., 1992, *Virology* 188:135-142), herpes simplex virus type 2 glycoprotein gB (Genbank accession no. M14923; Bzik et al., 198, *Virology* 155:322-333), poliovirus I VP1 (Emini et al., 1983, *Nature* 304:99), envelope glycoproteins of HIV I, such as gp120(Putney et al., 198, *Science* 234:1392-1395), hepatitis B surface antigen (Itoh et al.,

198, *Nature* 308:19; Neurath et al., 198, *Vaccine* 4:34), diptheria toxin (Audibert et al., 1981, *Nature* 289:543), streptococcus 24M epitope (Beachey, 1985, *Adv. Exp. Med. Biol.* 185:193), gonococcal pilin (Rothbard and Schoolnik, 1985, *Adv. Exp. Med. Biol.* 185:247), pseudorabies virus g50 (gpD), pseudorabies virus II (gpB), pseudorabies virus gIII (gpC), pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral diarrhea glycoprotein 55, newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, foot and mouth disease virus, hog colera virus, swine influenza virus, african swine fever virus, *Mycoplasma hypopneumoniae*, infectious bovine rhinotracheitis virus (*e.g.*, infectious bovine rhinotracheitis virus glycoprotein E or glycoprotein G), infectious laryngotracheitis virus (*e.g.*, infectious laryngotracheitis virus glycoprotein G or glycoprotein I), a glycoprotein of La Crosse virus (Gonzales-Scarano et al., 1982, *Virology* 120:42), neonatal calf diarrhea virus (Matsuno and Inouye, 1983, *Infection and Immunity* 39:155), Venezuelan equine encephalomyelitis virus (Mathews and Roehrig, 1982, *J. Immunol.* 129:273), punta toro virus (Dalrymple et al., 1981, in *Replication of Negative Strand Viruses*, Bishop and Compans (eds.), Elsevier, NY, p. 17), murine leukemia virus (Steeves et al., 1974, *J. Virol.* 14:187), mouse mammary tumor virus (Massey and Schochetman, 1981, *Virology* 115:20), hepatitis B virus core protein and/or hepatitis B virus surface antigen (see, *e.g.*, U.K. Patent Publication No. GB 2034323A published June 4, 1980; Ganem and Varmus, 1987, *Ann. Rev. Biochem.* 5:51-93; Tiollais et al., 1985, *Nature* 317:489-495), antigen of equine influenza virus or equine herpesvirus (*e.g.*, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, antigen of bovine respiratory syncytial virus or bovine parainfluenza virus (*e.g.*, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase), bovine viral diarrhea virus glycoprotein 48 or glycoprotein 53. In other specific embodiments, infectious diseases are treated or prevented by administration of a modified immunoglobulin (or functionally active fragment, derivative, or analog thereof, or nucleic acid encoding the same) that recognizes a cellular receptor for aninfectiusdisease agent, for example but not by way of

limitation, such cellular receptors, along with their corresponding pathogens are listed in Table 4.

Table 4

Pathogen	Cellular Receptor
B-lymphotropic papovavirus (LPV)	LPV receptor on B-cells
Bordatella pertussis	Adenylate cyclase
Borna Disease virus (BDV)	BDV surface glycoproteins
Bovine coronavirus	N-acetyl-9-O-acetylneuraminic acid receptor
Choriomeningitis virus	$CD4^+$
Dengue virus	Highly sulphated type heparin sulphate p65
E. coli	Gal-alpha-1-4Gal-containing isoreceptors
Ebola	CD16b
Echovirus 1	Integrin VLA-2 receptor
Echovirus-11 (EV)	EV receptor
Endotoxin (LPS)	CD14
Enteric bacteria	Glycoconjugate receptors
Enteric Orphan virus	alpha/beta T-cell receptor
Enteroviruses	Decay-accelerating factor receptor
Feline leukemia virus	Extracellular envelope glycoprotein (Env-SU) receptor
Foot and mouth disease virus	Immunoglobulin Fc receptor
Gibbon ape leukemia virus (GALV)	GALV receptor
Gram-negative bacteria	CD14 receptor
Heliobacter pylori	Lewis(b) blood group antigen receptor
Hepatitis B virus (HBV)	T-cell receptor

Pathogen	Cellular Recept r
Herpes Simplex Virus	Heparin sulphate glycosaminoglycan receptor Fibroblast growth factor receptor
HIV-1	CC-Chemokine receptor CCR5 CD11a CD2 G-protein coupled receptor CD4
Human cytomegalovirus	Heparin sulphate proteoglycan Annexin II CD13 (aminopeptidase N)
Human coronovirus	Human aminopeptidase N receptor
Influenza A, B & C	Hemagglutinin receptor
Legionella	CR3 receptor Protein kinase receptor Galactose N-acetylgalactosamine (Gal/GalNAc)-inhibitable lectin receptor Chemokine receptor
Leishmania mexicana	Annexin I
Listeria monocytogenes	ActA protein
Measles virus	CD46 receptor
Meningococcus	Meningococcal virulence associated Opa receptors
Morbilliviruses	CD46 receptor
Mouse hepatitis virus	Carcinoembryonic antigen family receptors Carcinoembryonic antigen family Bg1a receptor
Murine leukemia virus	Envelope glycoproteins
Murine gamma herpes virus	gamma interferon receptor
Murine retrovirus	Glycoprotein gp70 Rmc-1 receptor
Murine coronavirus mouse hepatitis virus	Carcinoembryonic antigen family receptors

Pathogen	Cellular Receptor
Mycobacterium avium-M	Human Integrin receptor alpha v beta 3
Neisseria gonorrhoeae	Heparin sulphate proteoglycan receptor CD66 receptor Integrin receptor Membrane cofactor protein CD46 GM1 GM2 GM3 CD3 Ceramide
Newcastle disease virus	Hemagglutinin-neuraminidase protein Fusion protein
Parvovirus B19	Erythrocyte P antigen receptor
Plasmodium falciparum	CD36 receptor Glycophorin A receptor
Pox Virus	Interferon gamma receptor
Pseudomonas	KDEL receptor
Rotavirus	Mucosal homing alpha4beta7 receptor
Samonella typhiurium	Epidermal growth factor receptor
Shigella	alpha5beta1 integrin protein
Streptococci	Nonglycosylated J774 receptor
T-helper cells type 1	Chemokine receptors including: 6. CXCR1-4 7. CCR1-5 8. CXCR3 9. CCR5
T-cell lymphotropic virus 1	gp46 surface glycoprotein

Pathogen	Cellular Receptor
Vaccinia virus	TNFRp55 receptor TNFRp75 receptor Soluble Interleukin-1 beta receptor

Viral diseases that can be treated or prevented by the methods of the present invention include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), any picornaviridae, enteroviruses, caliciviridae, any of the Norwalk group of viruses, togaviruses (such as Dengue virus), alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-, cercopithecine herpes virus 1 (B virus), poxviruses, and encephalitis.

Bacterial diseases that can be treated or prevented by the methods of the present invention are caused by bacteria including, but not limited to, gram negative and gram positive bacteria, mycobacteria rickettsia, mycoplasma, Neisseria spp. (*e.g.*, *Neisseria mennigitidis* and *Neisseria gonorrhoeae*), legionella, *Vibrio cholerae*, Streptococci, such as *Streptococcus pneumoniae*, *corynebacteria diphtheriae*, *clostridium tetani*, *bordetella pertussis*, Haemophilus spp. (*e.g.*, *influenzae*), Chlamydia spp., Enterotoxigenic *Escherichia coli*, *Shigella spp.* etc., and bacterial diseases such as Syphilis, Lyme's disease, etc.

Protozoal diseases that can be treated or prevented by the methods of the present invention are caused by protozoa including, but not limited to, plasmodia, eimeria, leishmania, kokzidioa, trypanosoma, fungi, such as candida, etc.

In specific embodiments of the invention, the Therapeutic of the invention is administered in conjunction with an appropriate antibiotic, anti-fungal, anti-viral or any other drug useful in treating or preventing the infectious disease.

5.3. GENE THERAPY

Gene therapy refers to treatment or prevention of a disease performed by the administration of a nucleic acid to a subject who has a disease associated with the expression of the antigen which is recognized by the immunoglobulin molecule from which the modified immunoglobulin molecule was derived. For example, the disease or disorder may be a cancer associated with the expression of a particular cancer or tumor agent or an infectious disease associated with the expression of a particular antigen of an infectious disease agent or for which the infectious disease agent binds a particular cellular receptor. In this embodiment of the invention, the therapeutic nucleic acid encodes a sequence that produces intracellularly (without a leader sequence) or intercellularly (with a leader sequence), a modified immunoglobulin.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY).

In one aspect, the therapeutic nucleic acid comprises an expression vector that expresses the modified immunoglobulin molecule.

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector or a delivery complex, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the antibodies. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in biopolymers (*e.g.*, poly-ß-1->4-N-acetylglucosamine polysaccharide; see

U.S. Patent No. 5,635,493), encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Young). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Alternatively, single chain antibodies, such as neutralizing antibodies, which bind to intracellular epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893). Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234. Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300).

The form and amount of therapeutic nucleic acid envisioned for use depends on the type of disease and the severity of its desired effect, patient state, etc., and can be determined by one skilled in the art.

5.3. VACCINE FORMULATIONS AND ADMINISTRATION

The invention also provides vaccine formulations containing Therapeutics of the invention, which vaccine formulations are suitable for administration to elicit a protective immune (humoral and/or cell mediated) response against certain antigens, *e.g.*, for the treatment and prevention of diseases.

Suitable preparations of such vaccines include injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection, may also be prepared. The preparation may also be emulsified, or the polypeptides encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, buffered saline, dextrose, glycerol, ethanol, sterile isotonic aqueous buffer or the like and combinations thereof. In addition, if desired, the vaccine preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Examples of adjuvants which may be effective, include, but are not limited to: aluminim hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine.

The effectiveness of an adjuvant may be determined by measuring the induction of anti-idiotype antibodies directed against the injected immunoglobulin formulated with the particular adjuvant.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, the lyophilized modified immunoglobulin of the invention is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Composition comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

The subject to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle) or any other standard routes of immunization. In a specific embodiment, scarification is employed.

The precise dose of the modified immunoglobulin molecule to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount sufficient to produce an immune response to the modified immunoglobulin molecule in the host (*i.e.*, an anti-idiotype reaction) to which the vaccine preparation is administered. Effective doses may also be extrapolated from dose-response curves derived from animal model test systems.

5.4. METHOD OF PRODUCING THE MODIFIED IMMUNOGLOBULINS

The modified immunoglobulins of the invention can be produced by any method known in the art for the synthesis of immunoglobulins, in particular, by chemical synthesis or by recombinant expression, and is preferably produced by recombinant expression techniques.

Recombinant expression of the modified immunoglobulin of the invention, or fragment, derivative or analog thereof, requires construction of a nucleic acid that encodes the modified immunoglobulin. If the nucleotide sequence of the modified immunoglobulin is known, a nucleic acid encoding the modified immunoglobulin may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the modified immunoglobulin, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR, *e.g.*, as exemplified in Section 6, *infra*.

Alternatively, the nucleic acid encoding the modified immunoglobulin may be generated from a nucleic acid encoding the immunoglobulin from which the modified immunoglobulin was derived. If a clone containing the nucleic acid encoding the particular immunoglobulin is not available, but the sequence of the immunoglobulin molecule is known, a nucleic acid encoding the immunoglobulin may be obtained from a suitable source (*e.g.*, an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by hybridization using an oligonucleotide probe specific for the particular gene sequence.

If an immunoglobulin molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an immunoglobulin is not available), immunoglobulins specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, *e.g.*, as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbon et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the immunoglobulin can be obtained by screening Fab expression libraries (*e.g.*, as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (see, *e.g.*, Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the immunoglobulin molecule is obtained, it may be introduced into any available cloning vector, and may be introduced into a vector containing the nucleotide sequence encoding the constant region of the immunoglobulin molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication

WO 89/01036; U.S. Patent No. 5,122,464; and Bebbington, 1991, Methods in Enzymology 2:136-145). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available, see *Id.* Then, the nucleic acid encoding the immunoglobulin can be modified to introduce the nucleotide substitutions or deletion necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group, along with any other desired amino acid substitutions, deletions or insertions. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical muagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can also be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constnat region derived from a human immunoglobulin, *e.g.*, humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., 1988, *Science* 242:1038-1041).

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments.

Once a nucleic acid encoding the modified immunoglobulin molecule of the invention has been obtained, the vector for the production of the immunoglobulin molecule may be produced by recombinant DNA technology using techniques well known in the art. The modified immunoglobulin molecule can then be recombinantly expressed and isolated

by any method known in the art, for example, using the method described in Section 6, *supra*, (see also Bebbington, 1991, Methods in Enzymology 2:136-145). Briefly, COS cells, or any other appropriate cultured cells, can be transiently or non-transiently transfected with the expression vector encoding the modified immunoglobulin, cultured for an appropriate period of time to permit immunoglobulin expression, and then the supernatan can be harvested from the COS cells, which supernatant contains the secreted, expressed modified immunoglobulin.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the immunoglobulin molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce the immunoglobulin of the invention.

The host cells used to express the recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli,* or, preferably, eukaryotic cells, especially for the expression of whole recombinant immunoglobulin molecules. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking et al., 198, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:2).

A variety of host-expression vector systems may be utilized to express the modified immunoglobulin molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the immunoglobulin molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing immunoglobulin coding sequences; insect cell systems infected with recombinant virus

expression vectors (*e.g.*, baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing immunoglobulin coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the immunoglobulin molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an immunoglobulin molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the immunoglobulin coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The immunoglobulin coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the immunoglobulin coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant

virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts (*e.g.*, see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals may also be required for efficient translation of inserted immunoglobulin coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:51-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the immunoglobulin molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the immunoglobulin molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the immunoglobulin molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}·nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The expression levels of the immunoglobulin molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *the Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing immunoglobulin is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the

amplified region is associated with the immunoglobulin gene, production of the immunoglobulin will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the modified immunoglobulin molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.5. DEMONSTRATION OF THERAPEUTIC UTILITY

The modified antibodies of the invention can be screened or assayed in a variety of ways for efficacy in treating or preventing a particular disease .

First, the immunopotency of a vaccine formulation containing the modified antibody of the invention can be determined by monitoring the anti-idiotypic response of test animals following immunization with the vaccine. Generation of a humoral response may be taken as an indication of a generalized immune response, other components of which, particularly cell-mediated immunity, may be important for protection against a disease. Test animals may include mice, rabbits, chimpanzees and eventually human subjects. A vaccine made in this invention can be made to infect chimpanzees experimentally. However, since chimpanzees are a protected species, the antibody response to a vaccine of the invention can first be studied in a number of smaller, less expensive animals, with the goal of finding one or two best candidate immunoglobulin molecules or best combinations of immunoglobulin molecules to use in chimpanzee efficacy studies.

The immune response of the test subjects can be analyzed by various approaches such as the reactivity of the resultant immune serum to antibodies, as assayed by known techniques, *e.g.*, enzyme linked immunosorbent assay (ELISA), immunoblots,

radioimmunoprecipitations, etc.; or protection from infection and/or attenuation of disease symptoms in immunized hosts.

As one example of suitable animal testing, the vaccine composition of the invention may be tested in rabbits for the ability to induce an anti-idiotypic response to the modified immunoglobulin molecule. For example, male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group of rabbits each receives an effective amount of the vaccine. A control group of rabbits receives an injection in 1 mM Tris-HCl pH 9.0 of the vaccine containing a naturally occurring antibody. Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for anti-idiotypic antibodies to the modified immunoglobulin molecule and anti-anti-idiotypic antibodies specific for the antigen against which the modified antibody was directed using, *e.g.*, a radioimmunoassay (Abbott Laboratories). The presence of anti-idiotypic antibodies may be assayed using an ELISA. Because rabbits may give a variable response due to their outbred nature, it may also be useful to test the vaccines in mice.

In addition, a modified antibody of the invention may be tested by first administering the modified antibody to a test subject, either animal or human, and then isolating the anti-anti-idiotypic antibodies (*i.e.*, the Ab3 antibodies) generated as part of the anti-idiotype response to the injected modified antibody. The isolated Ab3 may then be tested for the ability to bind the particular antigen (*e.g.*, a tumor antigen, antigen of an infectious disease agent by any immunoassays known in the art, for example, but not limited to, radioimmunoassays, ELISA, "sandwich" immunoassay, gel diffusion precipitin reactions, immunodiffusion assays, western blots, precipitation reactions, agglytination assays, complement fixation assays, immunofluorescence assays, protein A assays, immunoelectrophoresis assays, etc.

In one aspect where the modified antibody is directed against a cancer or tumor antigen, the efficacy of the isolated Ab3 for treating cancer, a tumor, or other neoplastic disease is screened by culturing cancer or tumor cells from a patient, contacting the cells with the Ab3 antibody to be tested, and comparing the proliferation or survival of the contacted cells with the proliferation or survival of cells not so contacted with the Ab3 antibody, wherein a lower level of proliferation or survival of the contacted cells indicates that the Ab3 antibody (which was elicited by immunization with the modified antibody of the invention) is effective to treat the cancer in the patient. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos, myc*) or cell

cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. If the modified antibody is directed against an antigen of an infectious disease agent, the isolated Ab3 may be tested for activity in any *in vitro* test for activity against the particular pathogen.

Additionally, the modified antibodies of the invention may also be tested directly *in vivo*. To monitor the effect of a Therapeutic of the invention, the level of the antigen against which the modified antibody is directed is measured at suitable time intervals before, during, or after therapy. Any change or absence of change in the amount of the antigen can be identified and correlated with the effect of the treatment on the subject.

In particular, in the case of cancer therapeutics, the serum levels of an antigen bears a direct relationship with severity of a cancer, such as breast cancer, and poor prognosis. Generally, a decrease in the level of antigen is associated with efficacious treatment.

When the modified antibody is directed against an antigen of an infectious disease agent, the efficacy of the modified antibody can be monitored by measuring the level of the antigen of the infectious disease agent at suitable times before, during and after therapy, where a decrease in the levels of the antigen indicates that the modified antibody is efficacious.

In a preferred aspect, the approach that can be taken is to determine the levels of antigen at different time points and to compare these values with a baseline level. The baseline level can be either the level of the marker present in normal, disease free individuals; and/or the levels present prior to treatment, or during remission of disease, or during periods of stability. These levels can then be correlated with the disease course or treatment outcome.

The levels of antigen can be determined by any method well known in the art. For example, a certain antigen can be quantitated by known immunodiagnostic methods such as western blotting immunoprecipitation using any antibody against a certain antigen.

The strength of the immune response *in vivo* to the modified immunogluobulin may be determined by any method known in the art, for example, but not limited to, delayed hypersensitivity skin tests and assays of the activity of cytolytic T-lymphocytes *in vitro*.

Delayed hypersensitivity skin tests are of great value in the testing of the overall immunocompetence and cellular immunity to an antigen. Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shortly before use. A 25- or 27-gauge need ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler.

Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

To test the activity of cytolytic T-lymphocytes, T-lymphocytes isolated from the immunized subject, *e.g.*, by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with cells bearing the antigen against which the modified antibody was directed in 3 ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2 is included in the culture medium as a source of T cell growth factors. In order to measure the primary response of cytolytic T-lymphocytes after immunization, the isolated T cells are cultured with or without the cells bearing the antigen. After six days, the cultures are tested for cytotoxity in a 4 hour ^{51}Cr-release assay. The spontaneous ^{51}Cr-release of the targets should reach a level less than 20% if immunization was effective (Heike et al., *J. Immunotherapy* 15:15-174).

In other aspects, the modified immunoglobulins may be tested for efficacy by monitoring the subject for improvement or recovery from the particular disease or condition associated with the antigen against which the modified antibody is directed. When the modified antibody is directed against a tumor or a cancer antigen, the progress of the particular tumor or cancer may be followed by any diagnostic or screening method known for monitoring cancer or a tumor. For example, but not by way of limitation, the cancer or tumor progress may be monitored by assaying the levels of the particular cancer or tumor antigen (or another antigen associated with the particular cancer or tumor) either in the serum of the subject or by injecting a labeled antibody specific for the antigen. Additionally, other imaging techniques, such as computed tomographic (CT) scan or sonograms, or any other imaging method, may be used to monitor the progression of the cancer or tumor. Biopsies may also be performed. Before carrying out such trials in humans, the tests for efficacy of the modified immunoglobulins can be performed in animal models of the particular cancer or tumor.

In the case of infectious diseases, the efficacy of the modified antibody can be assayed by administering the modified antibody to a subject (either a human subject or an animal model for the disease) and then monitoring either the levels of the particular infectious disease agent or symptoms of the particular infectious disease. The levels of the infectious disease agent may be determined by any method known in the art for assaying the levels of an infectious disease agent, *e.g.*, the viral titer, in the case of a virus, or bacterial levels (for example, by culturing of a sample from the patient), etc. The levels of the infectious disease agent may also be determined by measuring the levels of the antigen

against which the modified immunoglobulin was directed or another antigen of the infectious disease agent. A decrease in the levels of the infectious disease agent or an amelioration of the symptoms of the infectious disease indicates that the modified antibody is effective.

6. EXAMPLE: ANTI-IDIOTYPIC VACCINE INDUCER FOR COLON CANCER

This example describes the construction of a modified antibody derived from the monoclonal antibody MAb31.1 (hybridoma secreting Mab31.1 is available from the American Type Tissue Collection as accession No. HB12314). Mab31.1 recognizes an antigen expressed by human colon carcinomas. The modified antibody of the invention, based on Mab31.1, was engineered to have variable region cysteine residues of both the heavy and light chain variable regions substituted with alanine. Therefore, the resulting modified antibody, was missing intrachain disulfide bonds in either the heavy and light chain variable regions.

6.1. CONSTRUCTION OF A MODIFIED ANTIBODY

The strategy for construction of the modified antibody was to construct two engineered genes that encoded the heavy and light chain variable regions wherein specific cysteine residues, known to be important in intra-chain disulfide bonding , were altered to alanine. Alanine residues were substituted for the cysteine residues at positions 22 and 92 of the heavy chain variable region of the antibody derived from Mab31.1 or at positions 23 and 88 of the Mab31.1 light chain variable region of the antibody derived from Mab31.1. In order to construct these engineered genes, groups of olionucleotides were assembled (as discussed below) and inserted into an appropriate vector providing constant regions.

In order to construct variable region genes encoding CDRs lacking intrachain disulfide bonds, the following strategy was performed.

First, single strand oligonucleotides were annealed to create cohesive double stranded DNA fragments (as diagramed in Figure 10, Step 1). Specifically, oligonucleotides of about 80 bases in length corresponding to the sequences of interest with 20 base overlapping regions were synthesized using automated techniques of GenoSys Biotech Inc. The specific sequences of each of these oligonucleotides. The specific sequences of these oligonucleotides are presented in Figures 9A and 9B. Figure 9A list the group of ten oligos used in engineering a heavy chain variable region gene called 2CAVHCOL1.

2CAVHCOL1 lacked 2 cysteine residues as compared to the consensus heavy chain variable gene. Figure 9B lists the group of 12 oligos used in the engineering of the light chain variable region gene called 2CAVLCOL1. 2CAVLCOL1 lacked two cysteine residues as compared to the consensus light chain variable region gene. In order to combine the oligos into the desired gene, groups of 10 or 12 oligos were combined as described below and as presented in Figure 10, where the identities of oligos 1 to 10 indicated in Figure 10 are provided in Table 5. Prior to combining, each oligonucleotide was 5' phosphorylated as follows: 25µl of each oligo was incubated for 1 hour in the presence of T4 polynucleotide kinase and 50mM ATP at 37°C. The reactions were stopped by heating for 5 minutes at 70°C followed by ethanol precipitation. Once phosphorylated, complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, oligo 5 + oligo 6), as shown in Figure 10, were then mixed in sterile microcentrifuge tubes and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing resulted in short double strand DNA fragments with cohesive ends.

Next, the cohesive double stand DNA fragments were ligated into longer strands (Figure 10, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double strand DNA fragments were ligated in the presence of T4 DNA ligase and 10mM ATP for 2 hours in a water bath maintained at 16°C. Annealed oligo 1/10 was mixed with annealed oligo 2/9, and annealed oligo 3/8 was mixed with annealed oligo 4/7. The resulting oligos were labeled oligo 1/10/2/9 and oligo 3/8/4/7. Next, oligo 3/8/4/7 was ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 was then ligated to oligo 1/10/2/9 resulted in a full length variable region gene.

Alternatively, when groups of 12 oligos were used, the order of addition was: 1+12 = 1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9 = 1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7= full length variable region gene. The names of oligonucleotides used in construction of the engineered genes are listed in Table 5. The modified heavy chain variable region gene was denoted as 2CAVHCOL1. The modified light chain variable region gene was denoted as 2CAVLCOL1.

The resulting modified variable region genes were then purified by gel electrophoresis. To remove unligated excess of oligos and other incomplete DNA fragments, ligated product was run on 1% low melting agarose gel at constant 110 V for 2 hours. The major band containing full length DNA product was cut out and placed in a sterile 1.5 ml centrifuge tube. To release the DNA from the agarose, the gel slice was

digested with f3-Agrase I at 40°C for 3 hours. The DNA was recovered by precipitation with 0.3 M NaOAc and isopropanol at —20°C for 1 hour followed by centrifugation at 12,000 rpm for 15 minutes. The purified DNA pellet was resuspended in 50 µl of TE buffer, pH 8.0. The engineered variable region gene was then amplified by PCR. Specifically, 100 ng of the engineered variable region gene was mixed with 25mM dNTPs, 200 ng of primers and 5 U of high fidelity thermostable Pfu DNA polymerase in buffer. Resulting PCR product was analyzed on 1% agarose gel.

Each purified DNA corresponding to the engineered variable region gene was subsequently inserted into the pUC19 bacterial vector. pUC19, is a 2686 base pair, a high copy number *E. coli* plasmid vector containing a 54 base pair polylinker cloning site in lacZ and an Amp selection marker. In order to prepare the vector for insertion of the engineered variable region gene, 10µg of pUC19 was linearized with *Hinc II* (50 U) for 3 hours at 37°C resulting in a vector with blunt end sequence 5' GTC. To prevent self re-ligation, linear vector DNA was dephosphorylated with 25 U of calf intestine alkaline phosphatase (CIP) for 1 hour at 37°C. In order to insert the engineered variable region gene into the pUC19 vector, approximately 0.5 µg of dephosphorylated linear vector DNA was mixed with 3 µg of phosphorylated variable region gene in the presence of T4 DNA ligase (1000 U), and incubated at 16°C for 12 hours.

The bacterial vector containing the engineered variable region gene was then used to transform bacterial cells. Specifically, freshly prepared competent DH5-α cells, 50 µl, were mixed with 1 µg of pUC19 containing the engineered variable region gene and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette was pulsed at 2.5 kV/200 ohm/25 µF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media was added to each cuvette and cells were allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation was removed, diluted 1:100, then 100 µl plated onto LB plates containing ampicillin (Amp 40 µg/ml). The plates were incubated at 37°C overnight due to the presence of the Amp marker. Only transformants containing pUC19 vector grew on LB/Amp plates.

A single transformant colony was picked and grown overnight in a 3 ml LB/Amp sterile glass tube with constant shaking at 37°C. The plasmid DNA was isolated using Easy Prep columns (Pharmacia Biotech.) and suspended in 100 µl of TE buffer, pH 7.5. To confirm the presence of gene insert in pUC19, 25 µl of plasmid DNA from each colony was digested with a restriction endonuclease for 1 hour at 37°C, and was analyzed on a 1% agarose gel. By this method plasmid DNA containing gene insert was resistant to enzyme cleavage due to loss of restriction site (5'..GTCGAC.. 3') and migrated as closed circular

(CC) DNA, while those plasmids without insert were cleaved and migrated as linear (L) double strand DNA fragment on gel.

In order to confirm correct gene sequences of the engineered variable region genes and to eliminate the possibility of unwanted mutations generated during the construction procedure, DNA sequencing was performed using M13/pUC reverse primer (5'AACAGCTATGACCATG 3') for the clones as well as PCR gene products using 5' end 20 base primer (5' GAATT CATGGCTTG GGTGTG 3') on automated ABI 377 DNA Sequencer. All clones were confirmed to contain correct sequences.

Table 5. Construction of gene encoding modified antibodies containing CDRs from Mab 31.1

	Oligo1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo	Oligo 7	Oligo 8	Oligo 9	Oligo10
2CAVHC OLI	VHC1	VHC2	VHC3	VHC4	VHC5	VHC	VHC7	VHC8	VHC9	VHC10
2CAVLC OLI	VLC1	VLC2	VLC3	VLC4	VLCS	VLC	VLC7	VLC8	VLC9	VLC10

6.3. INSERTION OF THE ENGINEERED VARIABLE REGION GENE INTO A MAMMALIAN EXPRESSION VECTOR

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. A modified variable region genes 2CAVHCOL1 or 2CAVLCOL1 were inserted into vectors containing appropriate constant regions. Engineered variable region genes lacking cysteine residues in the light chain, were inserted into the pMRRO10.1 vector Figure 6A. The pMRRO10.1 vector contained a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gave a complete light chain sequence. Alternatively, the engineered variable region gene lacking cysteine residues in the heavy chain, were inserted into the pGAMMA1 vector Figure 6B. The pGAMMA1 vector contained human and IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector gave a complete heavy chain sequence.

In order to engineer a mammalian vector comprising both heavy chain and light chain genes, the complete light chain sequence and complete heavy chain sequence were inserted into mammalian expression vector pNEPuDGV as shown in Figure 6C (Bebbington, C.R., 1991, In METHODS: A Companion to Methods in Enzymology, vol. 2,

pp. 136-145). The resulting vector encoding both light chain and the heavy chain of the modified antibody.

6.4. EXPRESSION OF SYNTHETIC MODIFIED ANTIBODIES IN MAMMALIAN CELLS

To examine the production of assembled antibodies the mammalian expression vector was transfected into COS cells. COS cells (an African green monkey kidney cell line, CV-1, transformed with an origin-defective SV40 virus) were used for short-term transient expression of the synthetic antibodies because of their capacity to replicate circular plasmids containing an SV40 origin of replication to very high copy number. The antibody expression vector was transferred to C0S7 cells (obtained from the American Type Culture Collection). The transfected cells were grown in Dulbecco's modified Eagle's Medium and transfected with the expression vectors using calcium precipitation (Sullivan et al., *FEBS Lett.* 285:120-123, 1991). The transfected cells were cultured for 72 hours after which supernatants were collected. Supernatants from transfected COS cells were assayed using ELISA method for assembled IgG. ELISA involves capture of the samples and standards onto a 96-well plate coated with an anti-human IgG Fc. Bound assembled IgG was detected with an anti-human Kappa chain linked to horse radish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB). Color development was proportional to the amount of assembled antibody present in the sample.

6.5. MODIFIED ANTIBODY IMMUNOSPECIFICALLY BINDS TO HUMAN COLON CARCINOMA CELLS AND ANTIGENS PRODUCED BY THESE CELLS

The modified antibody was expressed and isolated as indicated in Section 6.4, *supra*. The binding capacity and specificity were then assayed using LS-174T cells WiDR cells (a human colon cancer cell line) and an antigen derived from these cells.

In order to examine the binding potency as well as specificity of MA31.1 binding, a dot blot analysis was performed (see Figure 11). Membrane preparations from LS-174T cells was applied to a nitrocellulose membrane using a Bio-Blot apparatus (Bio-Rad). The wells were blocked for non-specific binding using skim milk. Biotinylated antibody derived from Mab31.1 was incubated in all wells. Unlabelled antibody at concentrations of 0.003 to 20 nM was then applied to the nitrocellulose membrane and allowed to incubate. Unbound antibody was removed from the membrane by washing and a second antibody, alkaline phosphatase labeled antihuman IgG, was added. Finally, an alkaline phosphatase substrate was added which generated a dark purple precipitate, indicating the presence of bound

labeled antibody. Figure 11 provides the results of the dot blot analysis. The figure demonstrated that the labeled antibody bound to the LS-174T cells. Additionally, the unlabeled antibody competed with biotinylated antibody binding, indicating specificity of binding of the antibody derived from Mab31.1 to tumor cell antigens.

6.6. ANTI-IDIOTYPE RESPONSE

The effect on binding of modified antibody to LS-174T cells was examined in a competition binding assay. LS-174T cells are human colon carcinoma cells which express antigen recognized by the Mab31.1 antibody. Peptides containing the sequence of one of the CDRs of the Mab31.1 antibody were generated. These peptides, the modified antibody and the control antibody derived from Mab31.1 were administered to mice in order to generate antisera against the CDR regions of Mab31.1 and the antibodies. Blood samples from mice were drawn two weeks and four weeks following injection. Antisera from the immuized mice were used in binding competition assays presented in Figures 12A and B.

Antisera and biotinylated antibodies were assayed for their ability to bind LS-174T cells. As demonstrated in Figure 12A and B, antisera raised to the CDR3 and CDR4 peptides dramatically competed for control antibody (antibody derived from Mab31.1) binding to LS-174T cells. Additionally, antisera raised against CDR1 and CDR2 also significantly competed for control antibody binding to LS-174T cells. Additionally, antisera from nice injected with the 2CAVHCOL1 and 2CAVLCOL1 antibodies (*i.e.*, the modified antibodies having the cysteine to alanine change in the variable region) competed for binding with the biotinylated antibody derived from Mab31.1 better than antiserum from mice injected with the antibody derived from Mab31.1 (Figure 12B). This result indicates that administration of the antibodies having the cysteine to alanine change in the variable region elicit an anti-idiotype antibodies that recognize the colon carcinoma cell antigen better than antibodies with variable region intra-chain disulfide bonds.

Table 6. Biotin-Labeled Peptides Derived from CDR Sequences of Mab 31.1

Peptide ID	Sequence
COL311 L1	biotin-N-Thr-Ala-Lys-Ala-Ser-Gln-Ser-Val-Ser-Asn-Asp-Val-Ala
COL311 L2	biotin-N-Ile-Tyr-Tyr-Ala-Ser-Asn-Arg-Tyr-Thr
COL311 L3	biotin-N-Phe-Ala-Gln-Gln-Asp-Tyr-Ser-Ser-Pro-Leu-Thr
COL311 H1	biotin-N-Phe-Thr-Asn-Tyr-Gly-Met-Asn
COL311 H2	biotin-N-Ala-Gly-Trp-Ile-Asn-Thr-Tyr-Thr-Gly-Glu-Pro-Thr-Tyr-Ala-Asp-Asp-Phe-Lys-Gly
COL311 H3	biotin-N-Ala-Arg-Ala-Tyr-Tyr-Gly-Lys-Tyr-Phe-Asp-Tyr

EXAMPLE 7: PRODUCTION OF A SYNTHETIC MODIFIED ANTIBODY CONTAINING HMFG-1 SEQUENCE

Antiidiotype antibodies were constructed which immunospecifically bound to the HMFG-1 antibody. HMFG-1 was an antibody to known to bind polymorphic epithelial mucin (PEM) (Stewart et al., 1990, J Clin Oncol 8:1941-50; Kosmas et al., 1994, Cancer 73:3000-3010). The antigenic determinant of PEM with the sequence ProAspThrArgPro was inserted into the variable chain region by methods of the invention. This short sequence is a highly immunogenic region of human polymorphic epithelial mucin (Gendler et al., 1988, J. Biol. Chem. 263:12820-12823). Residues 27A-27E (SerLeuLeuTyrSer) of HMFG-1 (Table 6) were replaced with ProAspThrArgPro using the oligonucleotide method described in section 6 *supra*, also in Figure 10. Antiidiotype synthetic HMFG-1 antibodies were produced which immunospecifically bound to HMFG-1, using the known sequences for the variable regions of the light and heavy chains of the HMFG-1. The oligos were added in the order 1 + 8 = 1/8, 2 + 7 = 2/7, 3 + 6 = 3/6, 4 + 5 = 4/5, 1/8 + 2/7 = 1/8/2/7, 3/6 + 4/5 = 3/6/4/5, 1/8/2/7 + 3/6/4/5 = 1/8/2/7/3/6/4/5. Table 7 shows sequence comparison between HMFG-1 and various consensus CDR sequences. Information concerning HMFG-1 and related monoclonal antibodies is set forth in WO 09/05142 (Imperial Cancer Research Technology, Ltd.) and humanized HMFG-1 is set forth in WO 92/04380 (Unilever).

Polymerase chain reaction (PCR) were used to amplify the assembled sequence as shown in Figure 13. The engineered variable regions gene constructed to contain nucleotide sequence encoding HMFG-1 is shown in Figure 13. The engineered variable region gene was inserted into appropriate vectors for antibody production, such as the pNEPuDGV vector, as described in Section 6, *supra*. Other methods for constructing engineered genes

may be used, including but not limited to those methods described by Jayaraman et al., 1989, *Nucleic Acids Res.* 17:4403; Sandhu et al., 1992, *BioTechniques* 12:14; Barnett and Erfie, 1990, *H. Nucleic Acids Res* 18:3094; Ciccarelli et al., 1991, *Nucleic Acids Res* 19:6007; Michaels et al., 1992, *BioTechniques* 12:45, incorporated by reference herein.

Table 7. Sequence comparison between HMFG-1 antibody and various consensus CDR sequences

VH1 CDR1 Sequences

Residue	31	32	33	34	35	35A	35B
Human I	Ser	Tyr	Ala	Ile	Ser		
Human II	Ser	Tyr	Ser/Tyr	Trp	Ser	Trp	Asn
Human III	Ser	Tyr	Ala	Met	Ser		
Mouse IA	Ser	Gly	Tyr	Trp	Asn	Asn	Ser
Mouse IB	Ser	Tyr	Gly	Val	His	Val	Ser
Mouse IIA	Asp/Ser	Tyr	Tyr	Met	Asn	Asn	
Mouse IIB	Ser	Tyr	Trp	Met	His		
Mouse IIC	Asp/Ser	Thr	Tyr	Met	His		
Mouse IIIA	Asp/Ser	Phe/Tyr	Tyr	Met	Glu		
Mouse IIIB	Arg	Tyr	Trp	Met	Ser		
Mouse IIIC	Arn	Tyr	Trp	Met	Asn		
Mouse IIID	Ser	Tyr	Ala	Met	Ser		
Mouse VA	Ser	Tyr	Gly	Ile	Asn		
Mouse VB	Ser	Tyr	Gly	Leu	Tyr		
HMFG-1	Ala	Tyr	Trp	Ile	Glu		

VH1 CDR2 Sequences

Residue	50	51	52	52A	52B	52C	53	54	55	56	57	58	59	60	61	62	63	64	65
Human I	Trp	Ile	Asn	Pro	Tyr		Gly	Asn	Gly	Asp	Thr	Asn	Tyr	Ala	Gln	Lys	Phe	Gln	Gly
Human II	Arg	Ile	Tyr	Tyr	Arg	Ala	Tyr	Ser	Gly	Ser	Thr	Asp/ Asn	Tyr	Asn	Pro	Ser	Leu	Lys	Ser
Human III	Val	Ile	Ser	Gly	Lys	Thr	Asp	Gly	Gly	Ser	Thr	Tyr	Tyr	Ala	Asp	Ser	Val	Lys	Gly
Mouse 1A	Tyr	Ile	Ser	+			Tyr	Ser	Gly	Ser	Thr	Tyr	Tyr	Asn	Pro	Ser	Leu	Lys	Ser
Mouse 1B	Val	Ile					Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met	Ser
Mouse IIA	Asp	Ile	Asn	Pro			Gly	Asn	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly
Mouse IIB	Arg	Ile	Asp	Pro			Asn	Ser	Gly	Gly	Thr	Asn	Tyr	Asn	Glu	Lys	Phe	Lys	Ser
Mouse IIC	Arg	Ile	Asp	Pro			Ala	Asn	Gly	Asn	Thr	Lys	Tyr	Asp	Pro	Lys	Phe	Gln	Gly
Mouse IIIA	Ala	Ser	Arg	Asn	Lys	Ala	Asn	Asp	Tyr	Thr	Thr	Glu	Tyr	Ser	Ala	Ser	Val	Lys	Gly
Mouse IIIB	Glu	Ile	Asn	Pro	Lys	Ala	Asp	Ser	Ser	Thr	Ile	Aan	Tyr	Thr	Pro	Ser	Leu	Lys	Asp
Mouse IIIC	Glu	Ile	Arg	Leu	Lys	Ser	Asp	Asn	Tyr	Ala	Thr	His	Tyr	Ala	Glu	Ser	Val	Lys	Gly
Mouse IIID	Thr	Ile	Ser	Ser	Lys	Ser	Gly	Gly	Gly	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly
Mouse VA	Tyr	Ile	Asn	Pro			Gly	Asn	Gly	Tyr	Thr	Lys	Tyr	Asn	Glu	Lys	Phe	Lys	Gly
Mouse VB	Tyr	Ile	Ser	Ser			Ser	Ser	Ala	Tyr	Pro	Asn	Tyr	Ala	Gln	Lys	Phe	Gln	Gly
HMFG-1	Glu	Ile	Leu	Pro			Gly	Ser	Asn	Asn	Ser	Arg	Tyr	Asn	Glu	Lys	Phe	Lys	Gly

VH1 CDR3 sequences

Residue	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J	100K	101	102
Human I	Ala	Pro	Gly	Tyr	Gly	Ser	Gly	Gly	Gly	Cys	Tyr	Arg	Gly	Asp	Tyr	+	Phe	Asp	Tyr
Human II	Glu	Leu	Pro	Gly	Gly	Tyr	+	Gly	Asp	Asp	Tyr	Tyr	Tyr	+	+	Gly	Phe	Asp	Val
Human III	+	Arg	+	+	+	Ser	Leu	Ser	Gly	+	Tyr	Tyr	Tyr	Tyr	His	Tyr	Phe	Asp	Tyr
Mouse 1A	Gly	Gly	Tyr	Gly	Tyr	Gly	Tyr	Tyr	Tyr	Tyr	Asp	+	Tyr	Tyr	Tyr	Tyr	Phe	Asp	Tyr
Mouse 1B	Asp	Arg	Gly	Arg	Tyr	Tyr	Tyr	+	Ser	Gly	+	+	+	Tyr	Tyr	Ala	Met	Asp	Tyr
Mouse IIA	Gly	+	Tyr	Tyr	Ser	Ser	Ser	Tyr	Met	+	Ala	+	+	Tyr	Tyr	Ala	Phe	Asp	Tyr

Residue	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J	100K	101	102
Mouse IIB	Tyr	Tyr	Tyr	Gly	Gly	Ser	Ser	+	+	Val	Tyr	+		Tyr	Trp	Tyr	Phe	Asp	Tyr
Mouse IIC	Gly	Tyr	Tyr	Tyr	Tyr	Asp	Ser	+	Val	Gly				Tyr	Tyr	Ala	Met	Asp	Tyr
Mouse IIIA	Asp	Tyr	Tyr	Tyr	Gly	Ser	Ser	Tyr	Tyr	Glu	Gly	Pro	Val	Tyr	Trp	Tyr	Phe	Asp	Val
Mouse IIIB	Leu	Gly	Gly	Tyr	Gly	Tyr	Phe	Gly	Ser	Ser				Tyr	Tyr	Ala	Met	Asp	Tyr
Mouse IIIC	Gly	Gly	Tyr	Gly	Gly	+	Arg	Arg	Ser						+	Trp	Phe	Ala	Tyr
Mouse IIID	Gly	Gly	Tyr	Tyr	Tyr	Leu	+	Gly	Ser	Ala	Pro	Phe		Asp	Tyr	Ala	Met	Asp	Tyr
Mouse VA	Ser	Asn	Tyr	Tyr	Gly	Gly	Ser	Tyr	Tyr	Tyr	+	Phe	Ala	Tyr	Tyr	Tyr	Phe	Asp	Tyr
Mouse VB	Arg	Val	Ile	Ser	Arg	Tyr	Phe											Asp	Gly
HMFG-1	Ser	Tyr	Asp	Phe	Ala	Trp	Phe											Ala	Tyr

VL CDR1 Sequences

residues	24	25	26	27	27A	27B	27C	27D	27E	27F	28	29	30	31	32	33	34
Human kappa I	Arg	Ala	Ser	Gln	Ser	Leu	Val	+	+		Ser	Ile	Ser	Asn/Ser	Tyr	Leu	Ala
Human kappa II	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser	+	Asp	Gly	Asn/Asp	Asn/Thr	Tyr	Leu	+
Human kappa III	Arg	Ala	Ser	Gln	Ser						Val	Ser	Ser	Ser	Tyr	Leu	Ala
Human kappa IV	Lys	Ser	Ser	Gln	Ser	Val	Leu	Tyr	Ser	Ser	Asn	Asn	Lys	Asn	Tyr	Leu	Ala
Mouse kappa I	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asn	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	
Mouse kappa II	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser		Asn	Gly	Asn	Thr	Tyr	Leu	Gln
Mouse kappa III	Arg	Ala	Ser	Glu	Ser	Val	Asp	Ser			Tyr	Gly	Asn	Ser	Phe	Met	His
Mouse kappa IV	Ser	Ala	Ser	Ser	Ser						Val	Ser	Ser	Ser	Tyr	Leu	His
Mouse kappa V	Arg	Ala	Ser	Gln	Asp						Asp	Ile	Ser	Asn	Tyr	Leu	Asn
Mouse kappa VI	Ser	Ala	Ser	Ser								Ser	Val	Ser	Tyr	Met	His
Mouse kappa VII																	
HMFG-1	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Tyr	Ser	Ser	Asn	Gln	Lys	Ile	Tyr	Leu	Ala

VL CDR2 Sequences

residues	50	51	52	53	54	55	56
Human kappa I	Ala	Ala	Ser	Ser	Leu	Glu	Ser
Human kappa II	Leu	Val	Ser	Asn	Arg	Ala	Ser
Human kappa III	Gly	Ala	Ser	Ser	Arg	Ala	Thr
Human kappa IV	Trp	Ala	Ser	Thr	Arg	Glu	Ser
Mouse kappa I	Trp	Ala	Ser	Thr	Arg	Glu	Ser
Mouse kappa II	Lys	Val	Ser	Asn	Arg	Phe	Ser
Mouse kappa III	Ala	Ala	Ser	Asn	Leu	Glu	Ser
Mouse kappa IV	Arg	Thr	Ser	Asn	Leu	Ala	Ser
Mouse kappa V	Tyr	Ala	Ser	Arg	Leu	His	Ser
Mouse kappa VI	Asp	Thr	Ser	Lys	Leu	Ala	Ser
Mouse kappa VII							
HMFG-1	Trp	Ala	Ser	Thr	Arg	Glu	Ser

VL CDR3 Sequences

residues	89	90	91	92	93	94	95	95A	95B	95C	95D	95E	95F	96	97
Human kappa I	Gln	Gln	Tyr	+	Ser	Leu	Pro	Glu						Trp	Thr
Human kappa II	Met	Gln	Ala	Leu	Gln	+	Pro	Arg						+	Thr
Human kappa III	Gln	Gln	Tyr	Gly	Ser	Ser	Pro	Pro						Leu	Thr
Human kappa IV	Gln	Gln	Tyr	Tyr	Ser	Thr	Pro							+	Thr
Mouse kappa I	Gln	Asn	Asp	Tyr	Ser	Tyr	Pro							Leu	Thr
Mouse kappa II	Phe	Gln	Gly	Thr	His	Val	Pro	Pro						Tyr	Thr
Mouse kappa III	Gln	Gln	Ser	Asn	Glu	Asp	Pro	Pro						Trp	Thr
Mouse kappa IV	Gln	Gln	Trp	Ser	Ser	Tyr	Pro	+	Gly					Leu	Thr
Mouse kappa V	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Pro						Arg	Thr
Mouse kappa VI	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Pro	Met				Pro	Leu	Thr

residues	89	90	91	92	93	94	95	95A	95B	95C	95D	95E	95F	96	97
Mouse kappa VII	Leu	Gln	Tyr	Asp	Glu	Phe	Ala							Tyr	Thr
HMFG-1	Gln	Gln	Tyr	Tyr	Arg	Tyr	Pro							Arg	Thr

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

2. The vaccine composition according to claim 1, wherein said antigen is a tumor antigen.

3. The vaccine composition according to claim 2, wherein said antigen is a cancer antigen.

4. The vaccine composition according to claim 3, wherein said antigen is a polymorphic epithelial mucin antigen.

5. The vaccine composition according to claim 3, wherein said antigen is a human colon carcinoma-associated protein antigen.

6. The vaccine composition according to claim 3, wherein said antigen is a human colon carcinoma-associated carbohydrate antigen.

7. The vaccine composition according to claim 1, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 of the light chain variable region of said second immunoglobulin molecule.

8. The vaccine composition according to claim 1, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl

group is at a position corresponding to position 22 or 92 of the heavy chain variable region of said second immunoglobulin molecule.

9. The vaccine composition according to claim 1, 7 or 8, wherein said amino acid residue that does not have a sulfhydryl group is alanine.

10. The vaccine composition according to claim 1, wherein said second immunoglobulin molecule is Mab 31.1, Mab33.28. or Mab HMFG-1 and wherein said one or more amino acid substitutions include a substitution with alanine at position 23 and/or 88 of the light chain variable region.

11. The vaccine composition according to claim 1, wherein said second immunoglobulin molecule is Mab 31.1, Mab 33.28, or Mab HMFG-1 and wherein said one or more amino acid substitutions include a substitution with alanine at position 22 and/or 92 of the heavy chain variable region.

12. The vaccine composition according to claim 3, wherein said antigen is a human milk fat globule antigen.

13. The vaccine composition according to claim 3, wherein said antigen is an antigen of a cancer of the breast, ovary, uterus, prostate, bladder, lung, skin, colon, pancreas, gastrointestinal tract, B cells or T cells.

14. The vaccine composition according to claim 3, wherein said antigen is said antigen is selected from the group consisting of KS 1/4 pan-carcinoma antigen, ovarian carcinoma antigen, prostatic acid phosphate, prostate specific antigen, melanoma-associated antigen p97, melanoma antigen gp75, high molecular weight melanoma antigen, prostate specific membrane antigen, carcinoembryonic antigen, polymorphic epithelial mucin antigen, human milk fat globule antigen, colorectal tumor-associated antigen TAG-72, CO17-1A, GICA 19-9, CTA-1, LEA, Burkitt's lymphoma antigen-38.13, CD19, human B-lymphoma antigen-CD20, CD33, ganglioside GD2, ganglioside GD3, ganglioside GM2, ganglioside GM3, tumor-specific transplantation type of cell-surface antigen, oncofetal antigen-alpha-fetoprotein L6, human lung carcinoma antigen L20, human leukemia T cell antigen-Gp37, neoglycoprotein, sphingolipids, EGFR, HER2 antigen, polymorphic epithelial mucin, malignant human lymphocyte antigen-APO-1, I antigen M18, M39, SSEA-

1, VEP8, VEP9, Myl, VIM-D5, $D_1$56-22, TRA-1-85, C14, F3, AH6, Y hapten, Le^y, TL5, FC10.2, gastric adenocarcinoma antigen, CO-514, NS-10, CO-43, MH2, 19.9 found in colon cancer, gastric cancer mucins, T_5A_7, R_{24}, 4.2, G_{D3}, D1.1, OFA-1, G_{M2}, OFA-2, G_{D2}, M1:22:25:8, SSEA-3, SSEA-4 and T-cell receptor derived peptides.

15. The vaccine composition according to claim 1, wherein said antigen is an antigen of an infectious disease agent.

16. The vaccine composition according to claim 15, wherein said antigen is selected from the group consisting of influenza virus hemagglutinin, human respiratory syncytial virus G glycoprotein, core protein of Dengue virus, matrix protein of Dengue virus, measles virus hemagglutinin, herpes simplex virus type 2 glycoprotein gB, poliovirus I VP1, envelope glycoproteins of HIV I, hepatitis B surface antigen, diptheria toxin, streptococcus 24M epitope, gonococcal pilin, pseudorabies virus g50, pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral diarrhea glycoprotein 55, Newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, infectious bovine rhinotracheitis virus glycoprotein E, infectious laryngotracheitis virus glycoprotein G or glycoprotein I, a glycoprotein of La Crosse virus, neonatal calf diarrhea virus, hepatitis B virus core protein, hepatitis B virus surface antigen, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, bovine respiratory syncytial virus attachment protein, bovine respiratory syncytial virus fusion protein, bovine respiratory syncytial virus nucleocapsid protein, bovine parainfluenza virus type 3 fusion protein, bovine parainfluenza virus type 3 hemagglutinin neuraminidase, bovine viral diarrhea virus glycoprotein 48, and bovine diarrhea virus glycoprotein 53.

17. The vaccine composition according to claim 1, wherein said antigen is a cellular receptor for an infectious disease agent.

18. The vaccine composition according to claim 17, wherein said cellular receptor is selected from the group consisting of LPV receptor, adenylate cyclase, BDV

surface glycoproteins, N-acetyl-9-O-acetylneuraminic acid receptor, $CD4^+$, highly sulphated type heparin sulphate, p65, Gal alpha 1-4-Gal-containing isoreceptors, CD16b, integrin VLA-2 receptor, EV receptor, CD14, glycoconjugate receptors, alpha/beta T-cell receptor, decay-accelerating factor receptor, extracellular envelope glycoprotein receptor, immunoglobulin Fc receptor poxvirus M-T7, GALV receptor, CD14 receptor, Lewis(b) blood group antigen receptor, T-cell receptor, heparin sulphate glycoaminoglycans receptor, fibroblast growth factor receptor, CD11a, CD2, G-protein coupled receptor, CD4, heparin sulphate proteoglycan, annexin II, CD13 (aminopeptidase N), human aminopeptidase N receptor, hemagglutinin receptor, CR3 receptor, protein kinase receptor, galactose N-acetylgalactosamine-inhibitable lectin receptor, chemokine receptor, annexin I, actA protein, CD46 receptor, meningococcal virulence associated opa receptors, CD46 receptor, carcinoembryonic antigen family receptors, carcinoembryonic antigen family Bg1a receptor, gamma interferon receptor, glycoprotein gp70, rmc-1 receptor, human integrin receptor alpha v beta 3, heparin sulphate proteoglycan receptor, CD66 receptor, integrin receptor, membrane cofactor protein, CD46, GM1, GM2, GM3, CD3, ceramide, hemagglutinin-neuraminidase protein, erythrocyte P antigen receptor, CD36 receptor, glycophorin A receptor, interferon gamma receptor, KDEL receptor, mucosal homing alpha4beta7 receptor, epidermal growth factor receptor, alpha5beta1 integrin protein, non-glycosylated J774 receptor, CXCR1-4 receptor, CCR1-5 receptor, CXCR3 receptor, CCR5 receptor, gp46 surface glycoprotein, TNFR p55 receptor, TNFp75 receptor, soluble interleukin-1 beta receptor.

19. The vaccine composition according to claim 15 or 17, wherein said infectious disease agent is a bacterium.

20. The vaccine composition according to claim 19, wherein said bacterium is selected from the group consisting of mycobacteria rickettsia, mycoplasma, Neisseria spp., legionella, *Shigella spp.*, *Vibrio cholerae*, Streptococci, *corynebacteria diphtheriae*, *clostridium tetani*, *bordetella pertussis*, Haemophilus spp., Chlamydia spp., and *Escherichia coli* or causes Syphilis or Lyme's disease.

21. The vaccine composition according to claim 15 or17 , wherein said infectious disease agent is a virus.

22. The vaccine composition according to claim 21 , wherein said virus is selected from the group consisting of hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I, herpes simplex type II, rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I, human immunodeficiency virus type II, picornaviruses, enteroviruses, caliciviridae, Norwalk group of viruses, togaviruses, alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1, and poxviruses.

23. The vaccine composition according to claim 15 or 17 , wherein said infectious disease agent is a parasite.

24. The vaccine composition according to claim 23, wherein said parasite is selected from a group consisting of plasmodia, eimeria, leishmania, kokzidioa, and trypanosoma and fungi.

25. The vaccine composition according to claim 1, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

26. A vaccine composition comprising an amount of a fragment of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said fragment comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to the corresponding fragment of a second immunoglobulin molecule, said fragment of said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

27. The vaccine composition according to claim 26, in which said fragment is a single chain immunoglobulin.

28. The vaccine composition according to claim 26, in which said fragment is a Fab fragment, $(Fab')_2$ fragment, a heavy chain dimer, a light chain dimer, or a Fv fragment.

29. The vaccine composition according to claim 26, in which said fragment further comprises a constant region.

30. The vaccine composition according to claim 26, in which the variable region is from a mouse immunoglobulin, and the constant region is from a human immunoglobulin.

31. The vaccine composition according to claim 1, in which the variable region has framework regions from a human antibody and complementarily determining regions (CDRs) from a mouse immunoglobulin.

32. The vaccine composition according to claim 1 in which the first immunoglobulin molecule is linked via a covalent bond to an amino acid sequence of a protein selected from the group consisting of Il-2, Il-4, Il-5, Il-, Il-7, Il-10, γ-interferon or MHC-derived peptide, G-CSF, TNF, porin, NK cell antigen or cellular endocytosis receptor.

33. The vaccine composition according to claim 26 in which the fragment of the first immunoglobulin molecule is linked via a covalent bond to an amino acid sequence of a protein selected from the group consisting of Il-2, Il-4, Il-5, Il-, Il-7, Il-10, γ-interferon or MHC-derived peptide, G-CSF, TNF, porin, NK cell antigen or cellular endocytosis receptor.

34. A method of generating an anti-idiotype response in a subject comprising administering to said subject an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

35. The method according to claim 34 which further comprises isolating an antibody from said subject, said antibody recognizing the idiotype of said second immunoglobulin molecule and administering said antibody to a second subject.

36. The method according to claim 34, wherein said antigen is a tumor antigen.

37. The method according to claim 34, wherein said antigen is a cancer antigen.

38. The method according to claim 36, wherein said antigen is a polymorphic epithelial mucin antigen.

39. The method according to claim 36, wherein said antigen is a human colon carcinoma-associated protein antigen.

40. The method according to claim 36, wherein said antigen is a human colon carcinoma-associated carbohydrate antigen.

41. The method according to claim 35, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 of the light chain variable region of said second immunoglobulin molecule.

42. The method according to claim 35, wherein said variable domain is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at position corresponding to position 22 or 92 of the heavy chain variable region of said second immunoglobulin molecule.

43. The method according to claim 34, wherein said amino acid residue that does not have a sulfhydryl group is alanine.

44. The method according to claim 34, wherein said second immunoglobulin molecule is Mab 31.1, Mab 33.28, and HMFG-1 and wherein said amino acid substitutions include a substitution with alanine at position 23 and/or 88 of the light chain variable region.

45. The method according to claim 34, wherein said second immunoglobulin molecule is Mab 31.1, Mab 33.28, and HMFG-1 and wherein said amino acid substitutions include a substitution with alanine at position 22 and/or 92 of the heavy chain variable region.

46. The method according to claim 34, wherein said antigen is a human milk fat globule antigen.

47. The method according to claim 34, wherein said antigen is an antigen for a cancer of the breast, ovary, uterus, prostate, bladder, lung, skin, pancreas, colon, gastrointestinal tract, B cell or T cell.

48. The method according to claim 36, wherein said antigen is selected from the group consisting of KS 1/4 pan-carcinoma antigen, ovarian carcinoma antigen, prostatic acid phosphate, prostate specific antigen, melanoma-associated antigen p97, melanoma antigen gp75, high molecular weight melanoma antigen, prostate specific membrane antigen, carcinoembryonic antigen, polymorphic epithelial mucin antigen, human milk fat globule antigen, colorectal tumor-associated antigen TAG-72, CO17-1A, GICA 19-9, CTA-1, LEA, Burkitt's lymphoma antigen-38.13, CD19, human B-lymphoma antigen-CD20, CD33, ganglioside GD2, ganglioside GD3, ganglioside GM2, ganglioside GM3, tumor-specific transplantation type of cell-surface antigen, oncofetal antigen-alpha-fetoprotein L6, human lung carcinoma antigen L20, human leukemia T cell antigen-Gp37, neoglycoprotein, sphingolipids, EGFR, HER2 antigen, polymorphic epithelial mucin, malignant human lymphocyte antigen-APO-1, I antigen M18, M39, SSEA-1, VEP8, VEP9, Myl, VIM-D5, $D_1$56-22, TRA-1-85, C14, F3, AH6, Y hapten, Le^y, TL5, FC10.2, gastric adenocarcinoma antigen, CO-514, NS-10, CO-43, MH2, 19.9 found in colon cancer, gastric cancer mucins, T_5A_7, R_{24}, 4.2, G_{D3}, D1.1, OFA-1, G_{M2}, OFA-2, G_{D2}, M1:22:25:8, SSEA-3, SSEA-4, and T-cell receptor derived peptides.

49. The method according to claim 34, wherein said antigen is an antigen of an infectious disease agent.

50. The method according to claim 49, wherein said antigen is selected from the group consisting of influenza virus hemagglutinin, human respiratory syncytial virus G glycoprotein, core protein of Dengue virus, matrix protein of Dengue virus, measles virus

hemagglutinin, herpes simplex virus type 2 glycoprotein gB, poliovirus I VP1, envelope glycoproteins of HIV I, hepatitis B surface antigen, diptheria toxin, streptococcus 24M epitope, gonococcal pilin, pseudorabies virus g50, pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral diarrhea glycoprotein 55, Newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, infectious bovine rhinotracheitis virus glycoprotein E, infectious laryngotracheitis virus glycoprotein G or glycoprotein I, a glycoprotein of La Crosse virus, neonatal calf diarrhea virus, hepatitis B virus core protein, hepatitis B virus surface antigen, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, bovine respiratory syncytial virus attachment protein, bovine respiratory syncytial virus fusion protein, bovine respiratory syncytial virus nucleocapsid protein, bovine parainfluenza virus type 3 fusion protein, bovine parainfluenza virus type 3 hemagglutinin neuraminidase, bovine viral diarrhea virus glycoprotein 48, and bovine diarrhea virus glycoprotein 53.

51. The method according to claim 34, wherein said antigen is a cellular receptor for an infectious disease agent.

52. The method according to claim 51, wherein said cellular receptor is selected from the group consisting of LPV receptor, adenylate cyclase, BDV surface glycoproteins, N-acetyl-9-O-acetylneuraminic acid receptor, $CD4^+$, highly sulphated type heparin sulphate, p65, Gal alpha 1-4-Gal-containing isoreceptors, CD16b, integrin VLA-2 receptor, EV receptor, CD14, glycoconjugate receptors, alpha/beta T-cell receptor, decay-accelerating factor receptor, extracellular envelope glycoprotein receptor, immunoglobulin Fc receptor poxvirus M-T7, GALV receptor, CD14 receptor, Lewis(b) blood group antigen receptor, T-cell receptor, heparin sulphate glycoaminoglycans receptor, fibroblast growth factor receptor, CD11a, CD2, G-protein coupled receptor, CD4, heparin sulphate proteoglycan, annexin II, CD13 (aminopeptidase N), human aminopeptidase N receptor, hemagglutinin receptor, CR3 receptor, protein kinase receptor, galactose N-acetylgalactosamine-inhibitable lectin receptor, chemokine receptor, annexin I, actA protein, CD46 receptor, meningococcal virulence associated opa receptors, CD46 receptor, carcinoembryonic antigen family

receptors, carcinoembryonic antigen family Bg1a receptor, gamma interferon receptor, glycoprotein gp70, rmc-1 receptor, human integrin receptor alpha v beta 3, heparin sulphate proteoglycan receptor, CD66 receptor, integrin receptor, membrane cofactor protein, CD46, GM1, GM2, GM3, CD3, ceramide, hemagglutinin-neuraminidase protein, erythrocyte P antigen receptor, CD36 receptor, glycophorin A receptor, interferon gamma receptor, KDEL receptor, mucosal homing alpha4beta7 receptor, epidermal growth factor receptor, alpha5beta1 integrin protein, non-glycosylated J774 receptor, CXCR1-4 receptor, CCR1-5 receptor, CXCR3 receptor, CCR5 receptor, gp46 surface glycoprotein, TNFR p55 receptor, TNFp75 receptor, soluble interleukin-1 beta receptor.

53. The method according to claim 49 or 51, wherein said infectious disease agent is a bacterium.

54. The method according to claim 53, wherein said bacterium is selected from the group consisting of mycobacteria rickettsia, mycoplasma, Neisseria spp., legionella, *Vibrio cholerae, Shigella spp.*, Streptococci, *corynebacteria diphtheriae, clostridium tetani, bordetella pertussis*, Haemophilus spp., Chlamydia spp., and *Escherichia coli*, or causes Syphilis or Lyme's disease.

55. The method according to claim 49 or 51, wherein said infectious disease agent is a virus.

56. The method according to claim 53, wherein said virus is selected from the group consisting of hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I, herpes simplex type II, rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I, human immunodeficiency virus type II, picornaviruses, enteroviruses, caliciviridae, Norwalk group of viruses, togaviruses, alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1, and poxviruses.

57. The method according to claim 49 or 51, wherein said infectious disease agent is a parasite.

58. The method according to claim 57, wherein said infectious paraste is selected from a group consisting of plasmodia, eimeria, leishmania, kokzidioa, and trypanosoma and fungi.

59. The method according to claim 34, in which said first immunoglobulin is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

60. A method of generating an anti-idiotype response in a subject comprising administering an amount of a fragment of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said fragment comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a corresponding fragment of a second immunoglobulin molecule, said fragment of said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

61. The method according to claim 60 which further comprises isolating an antibody from said subject, said antibody recognizing the idiotype of said second immunoglobulin molecule and administering said antibody to a second subject.

62. The method according to claim 60, wherein said antigen is a tumor antigen.

63. The method according to claim 60, wherein said antigen is a cancer antigen.

64. The method according to claim 60, wherein said antigen is a polymorphic epithelial mucin antigen.

65. The method according to claim 62, wherein said antigen is a human colon carcinoma-associated protein antigen.

66. The method according to claim 62, wherein said antigen is a human colon carcinoma-associated carbohydrate antigen.

67. The method according to claim 60, wherein said variable region is a light chain variable region and said amino acid residue that does not have a sulfhydryl group at position corresponding to position 23 or 88 of the heavy chain variable region of said second immunoglobulin molecule.

68. The method according to claim 60, wherein said variable domain is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at position corresponding to position 22 or 92 of the heavy chain variable region of said second immunoglobulin molecule.

69. The method according to claim 60, 67, or 68, wherein said amino acid residue that does not have a sulfhydryl group is alanine.

70. The method according to claim 63, wherein said antigen is a human milk fat globule.

71. The method according to claim 63, wherein said antigen is an antigen for a cancer of the breast, ovary, uterus, prostate, bladder, lung, skin, pancreas, colon, gastrointestinal tract, B cell or T cell.

72. The method according to claim 60, wherein said antigen is an antigen of an infectious disease agent.

73. The method of claim 72, wherein said antigen is selected from the group consisting of influenza virus hemagglutinin, human respiratory syncytial virus G glycoprotein, core protein of Dengue virus, matrix protein of Dengue virus, measles virus hemagglutinin, herpes simplex virus type 2 glycoprotein gB, poliovirus I VP1, envelope glycoproteins of HIV I, hepatitis B surface antigen, diptheria toxin, streptococcus 24M epitope, gonococcal pilin, pseudorabies virus g50, pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral

diarrhea glycoprotein 55, Newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, infectious bovine rhinotracheitis virus glycoprotein E, infectious laryngotracheitis virus glycoprotein G or glycoprotein I, a glycoprotein of La Crosse virus, neonatal calf diarrhea virus, hepatitis B virus core protein, hepatitis B virus surface antigen, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, bovine respiratory syncytial virus attachment protein, bovine respiratory syncytial virus fusion protein, bovine respiratory syncytial virus nucleocapsid protein, bovine parainfluenza virus type 3 fusion protein, bovine parainfluenza virus type 3 hemagglutinin neuraminidase, bovine viral diarrhea virus glycoprotein 48, and bovine diarrhea virus glycoprotein 53.

74. The method according to claim 60, wherein said antigen is a cellular receptor for an infectious disease agent.

75. The method according to claim 74, wherein said cellular receptor is selected from the group consisting of LPV receptor, adenylate cyclase, BDV surface glycoproteins, N-acetyl-9-O-acetylneuraminic acid receptor, $CD4^+$, highly sulphated type heparin sulphate, p65, Gal alpha 1-4-Gal-containing isoreceptors, CD16b, integrin VLA-2 receptor, EV receptor, CD14, glycoconjugate receptors, alpha/beta T-cell receptor, decay-accelerating factor receptor, extracellular envelope glycoprotein receptor, immunoglobulin Fc receptor poxvirus M-T7, GALV receptor, CD14 receptor, Lewis(b) blood group antigen receptor, T-cell receptor, heparin sulphate glycoaminoglycans receptor, fibroblast growth factor receptor, CD11a, CD2, G-protein coupled receptor, CD4, heparin sulphate proteoglycan, annexin II, CD13 (aminopeptidase N), human aminopeptidase N receptor, hemagglutinin receptor, CR3 receptor, protein kinase receptor, galactose N-acetylgalactosamine-inhibitable lectin receptor, chemokine receptor, annexin I, actA protein, CD46 receptor, meningococcal virulence associated opa receptors, CD46 receptor, carcinoembryonic antigen family receptors, carcinoembryonic antigen family Bg1a receptor, gamma interferon receptor, glycoprotein gp70, rmc-1 receptor, human integrin receptor alpha v beta 3, heparin sulphate proteoglycan receptor, CD66 receptor, integrin receptor, membrane cofactor protein, CD46, GM1, GM2, GM3, CD3, ceramide, hemagglutinin-neuraminidase protein, erythrocyte P antigen receptor, CD36 receptor, glycophorin A receptor, interferon gamma receptor, KDEL receptor, mucosal homing alpha4beta7 receptor, epidermal growth factor receptor,

alpha5beta1 integrin protein, non-glycosylated J774 receptor, CXCR1-4 receptor, CCR1-5 receptor, CXCR3 receptor, CCR5 receptor, gp46 surface glycoprotein, TNFR p55 receptor, TNFp75 receptor, soluble interleukin-1 beta receptor.

76. The method according to claim 72 or 74, wherein said infectious disease agent is a bacterium.

77. The method according to claim 76, wherein said bacterium is selected from the group consisting of mycobacteria rickettsia, mycoplasma, Neisseria spp., legionella, *Vibrio cholerae, Shigella spp.*, Streptococci, *corynebacteria diphtheriae*, *clostridium tetani*, *bordetella pertussis*, Haemophilus spp., Chlamydia spp., and *Escherichia coli*, or causes Syphilis or Lyme's disease.

78. The method according to claim 72 or 74, wherein said infectious disease agent is a virus.

79. The method according to claim 78, wherein said virus is selected from the group consisting of hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I, herpes simplex type II, rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I, human immunodeficiency virus type II, picornaviruses, enteroviruses, caliciviridae, Norwalk group of viruses, togaviruses, alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1, and poxviruses.

80. The method according to claim 72 or74, wherein said infectious disease agent is a parasite.

81. The method according to claim 80, wherein said parasite is selected from a group consisting of plasmodia, eimeria, leishmania, kokzidioa, trypanosoma, and fungi.

82. The method according to claim 60, in which said fragment is a single chain immunoglobulin.

83. The method according to claim 82, in which the fragment is a Fab fragment, $(Fab')_2$ fragment, a heavy chain dimer, a light chain dimer, or an Fv fragment.

84. The method according to claim 60, in which said fragment further comprises a constant region.

85. A method according to claim 84, in which the variable region is from a mouse immunoglobulin, and the constant region is from a human immunoglobulin.

86. A method according to claim 60, in which the variable region has framework regions from a human antibody and complementarily determining regions (CDRs) from a mouse immunoglobulin.

87. A method of treating or preventing cancer, in a subject in need of such treatment or prevention, said method comprising administering to a subject a vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding a cancer antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

88. A method of treating or preventing an infectious disease, in a subject in need of such treatment or prevention, said method comprising administering to a subject a vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen of an infectious disease agent, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a

sulfhydryl group at one or more positions corresponding to one or more cysteine residues that forms a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

89. A method of treating or preventing an infectious disease, in a subject in need of such treatment or prevention, said method comprising administering to a subject a vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding a cellular receptor for an infectious disease agent, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that forms a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

90. The method according to claim 88 or 89 , in which said infectious disease is selected from the group consisting of Syphilis, gonorrhea, AIDS, malaria, shigella, salmonella, hepatitis A, hepatitis C, lyme disease, encephalitis, herpes, gram negative bacterial infection, gram positive bacterial infection, and pneumococcus infection.

91. A method of preparing a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binds an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule, said method comprising the steps of:

(a) constructing a nucleic acid that encodes said first immunoglobulin molecule by replacing in a nucleic acid that encodes said second immunoglobulin the nucleotides encoding said one or more cysteine residues that form said disulfide bond with the nucleotides that encode said one or more amino acid residues that do not have a sulhydyl group;

(b) introducing the nucleic acid constructed in step (a) into a cell such that said first immunoglobulin molecule is expressed by the cell; and

(c) recovering the expressed first immunoglobulin molecule.

92. The method of claim 91 wherein said nucleotides are replaced by site-directed mutagenesis.

93. A method of preparing a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable domain and being identical, except for one or more amino acid substitutions in said variable domain, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binds an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule, comprising the steps of:

(a) synthesizing a nucleic acid containing an artificial gene that encodes said first immunoglobulin molecule;

(b) introducing the nucleic acid synthesized in step (a) into a cell such that the encoded first immunoglobulin molecule is expressed by the cell; and

(c) recovering the expressed first immunoglobulin molecule.

94. The method of claim 91 or 93, in which said second immunoglobulin molecule is Mab 31.1 or Mab 33.28.

95. The method of claim 91 or 93, in which said second immunoglobulin molecule is HMFG-1.

96. A vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, in which at least some of said one or more amino acid substitutions are the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second

immunoglobulin molecule, wherein the amino acid substitutions that are not the substitution of one or more amino acid residues that do not have a sulfhydryl group at said one or more positions corresponding to said one or more cysteine residues that form said disulfide bond in said second immunoglobulin molecule are not stabilizing changes; and a pharmaceutically acceptable carrier.

97. A method of generating an anti-idiotype response in a subject comprising administering to said subject an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, in which at least some of said one or more amino acid substitutions are the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule, wherein the amino acid substitutions that are not the substitution of one or more amino acid residues that do not have a sulfhydryl group at said one or more positions corresponding to said one or more cysteine residues that form said disulfide bond in said second immunoglobulin molecule are not stabilizing changes.

98. A first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, in which at least some of said one or more amino acid substitutions are the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule, wherein the amino acid substitutions that are not the substitution of one or more amino acid residues that do not have a sulfhydryl group at said one or more positions corresponding to said one or more cysteine residues that form said disulfide bond in said second immunoglobulin molecule are not stabilizing changes.

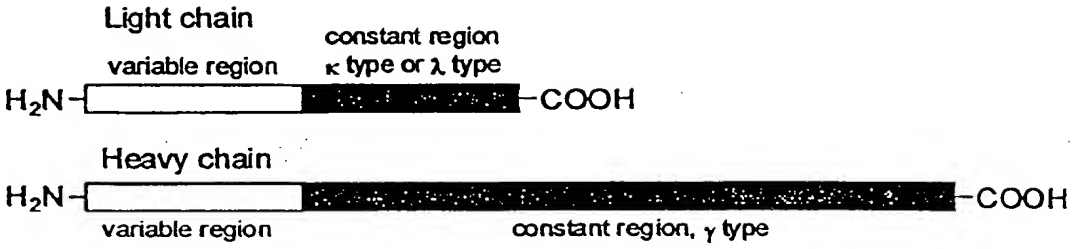

Light chain
variable region
constant region
κ type or λ type
H2N
COOH
Heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

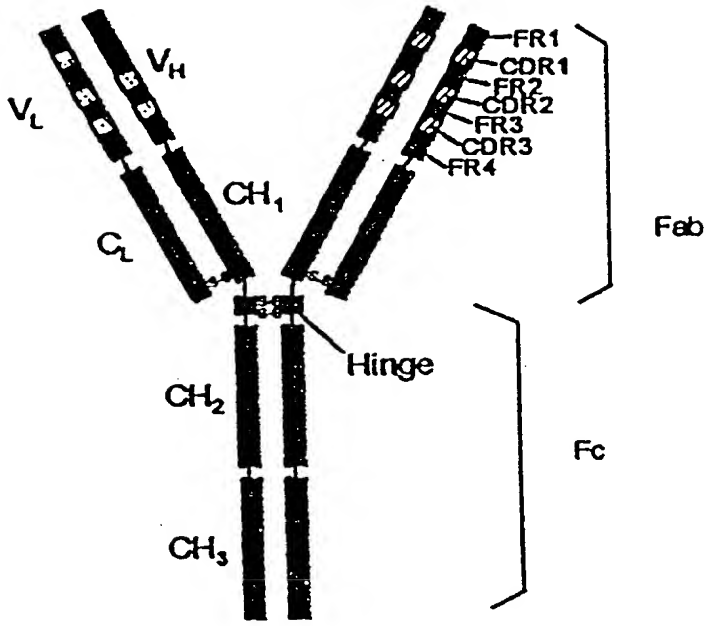


FIG. 2

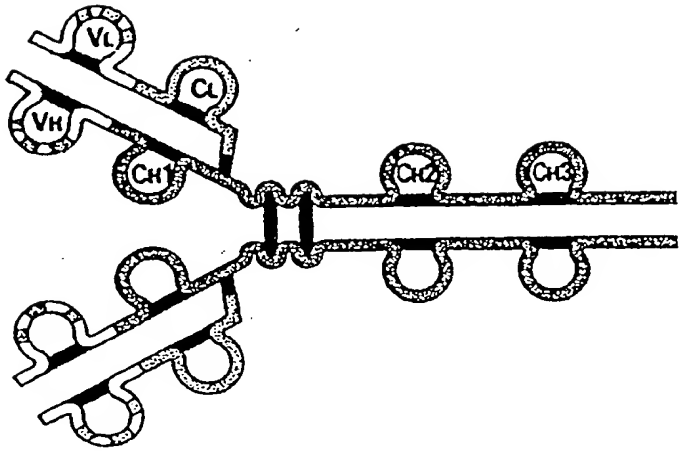


FIG. 3

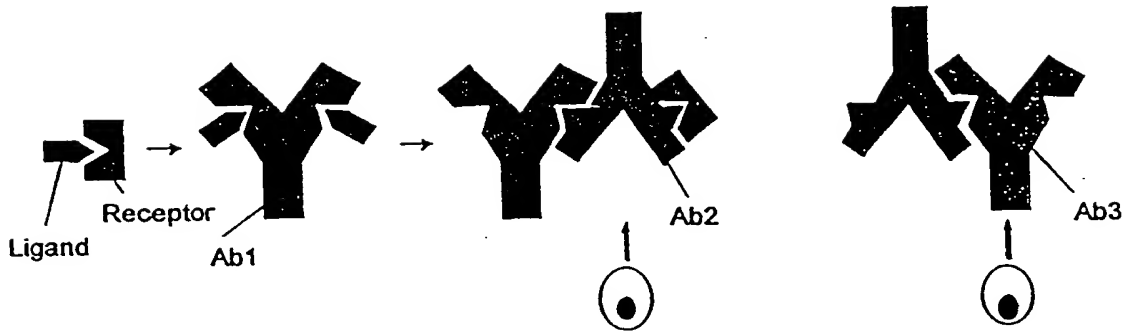


FIG. 4

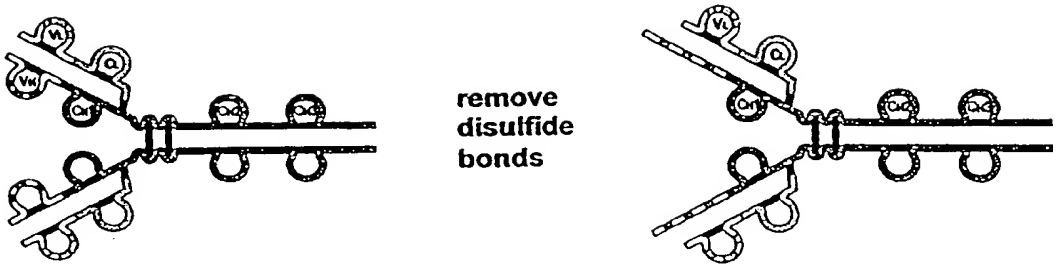


FIG. 5

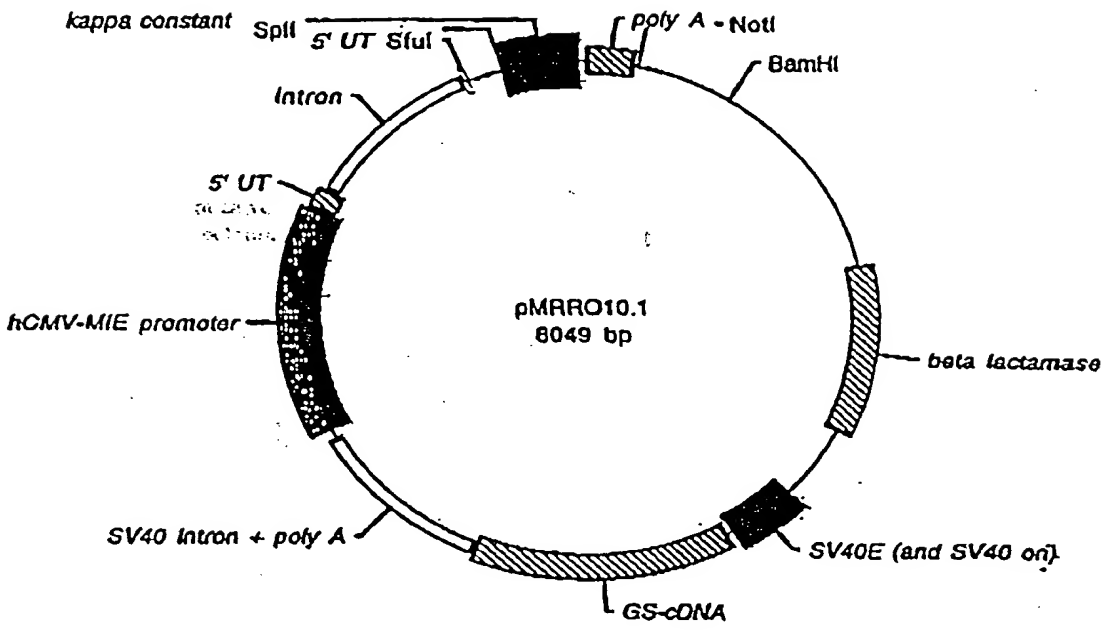

kappa constant
SpII
5' UT SfuI
poly A - NotI
BamHI
Intron
5' UT
pMRRO10.1
6049 bp
hCMV-MIE promoter
beta lactamase
SV40 intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 6A

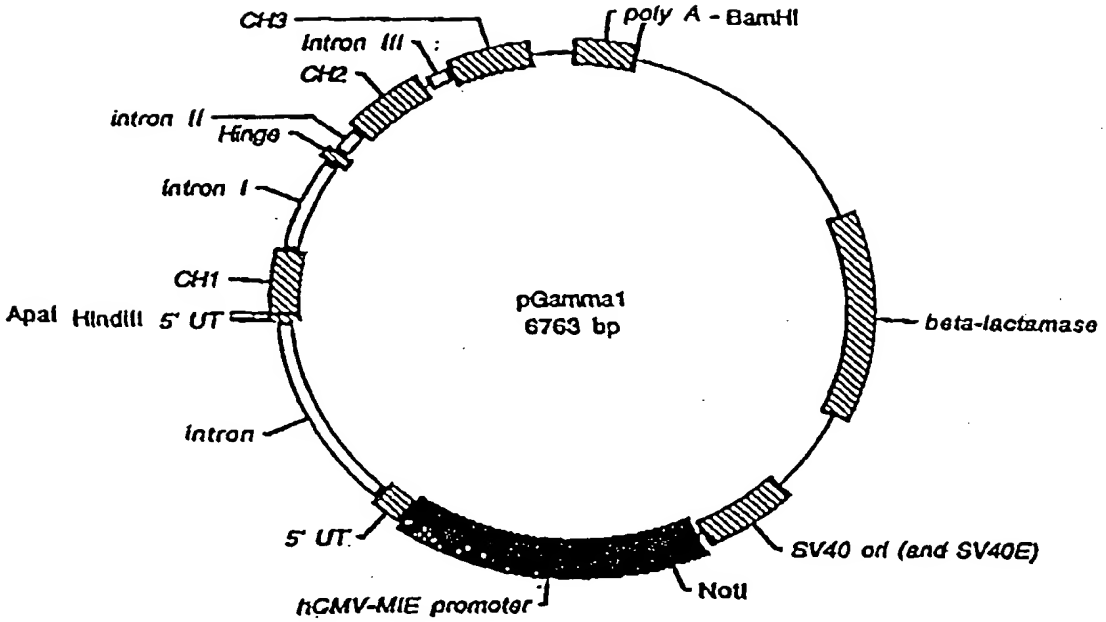

CH3
Intron III
CH2
intron II
Hinge
intron I
CH1
ApaI HindIII 5' UT
intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 6B

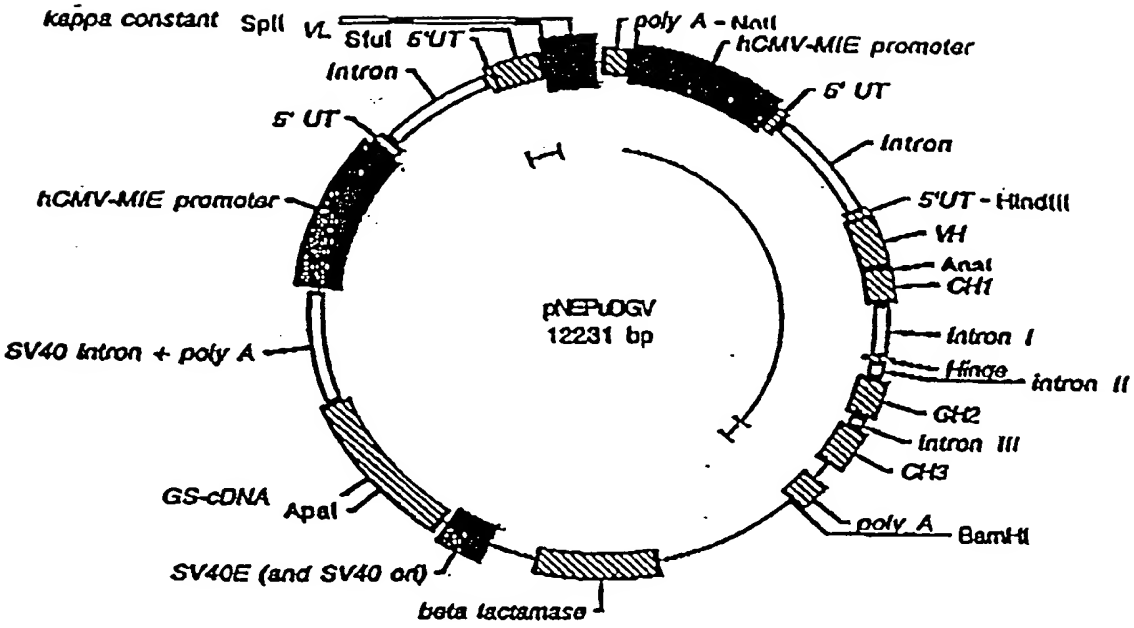

kappa constant
SpII
VL
SfuI 5'UT
Intron
5' UT
hCMV-MIE promoter
SV40 intron + poly A
GS-cDNA
ApaI
SV40E (and SV40 ori)
beta lactamase
poly A - NotI
hCMV-MIE promoter
5' UT
Intron
5'UT - HindIII
VH
ApaI
CH1
Intron I
Hinge
Intron II
CH2
Intron III
CH3
poly A
BamHI
pNEPuDGV
12231 bp

FIG. 6C

ConVL1

EcoR1
GAA TTC
6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
Eco R1

FIG. 7A

```
ConVH1

EcoR1                                                              6
GAA TTC

-19 (Leader)                                                      -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA            63

VL:
1                          10                          20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG        123


21                         30                          35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT            189

41                         50                          60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC        249

61                         70                          80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG        309

81       82A 82B 82C                        90
        100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT        378

101                        110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
       423
```

FIG. 7B

```
2CAVLCOL1
EcoR1                                                          6
GAA TTC                                                      -19  (Leader)
                                  -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA        63

1                                        10                                  20
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly Asp Arg Val Thr
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTT ACC    123

21                                       30                                  40
Ile Thr Ala Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro
ATA ACC GCT AAG GCC AGT CAG AGT GTG AGT AAT GAT GTA GCT TGG TAC CAA CAG AAA CCA    183

41                                       50                                  60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT    243

61                                       70                                  80
Arg Phe thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser thr Val Gln Ala
CGC TtC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT    303

81                                       90                                 100
Glu Asp Leu Ala Val Tyr Phe Ala Gln Gln Asp Tyr Ser Ser Pro Leu Thr Phe Gly Ala
GAA GAC CTG GCA GTT TAT TTC GCT CAG CAG GAT TAT AGC TCT CCG CTC ACG TTC GGT GCT    363

101
Gly Thr Lys Leu Glu Leu Lys
GGG ACC AAG CTG GAG CTG AAA GAA TTC                                              390
                        EcoR1
```

FIG. 8A

```
2CAVHCOL1
EcoR1
GAA TTC                                                                  6

-19 (Leader)                                                        -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA      63

1                                        10                                  20
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr Val Lys Ile
CAG ATC CAG TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG ACA GTC AAG ATC     123

21                                       30                                  40
Ser Ala Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala
TCC GCT AAG GCT TCT GGG TAT ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT     183

41                                       50                                  60
Pro Gly Lys Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
CCA GGA AAG GGT TTA AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT     243

61                                       70                                  80
Ala Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
GCT GAT GAC TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC AGC ACT GCC TAT     303

81                                       90                                 100
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Ala Ala Arg Ala Tyr
TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT TTC GCT GCA AGA GCC TAC     363

101
Tyr Gly Lys Tyr Phe Asp Tyr
TAT GGT AAA TAC TTT GAC TAC GAA TTC                                              390
```

FIG. 8B

2CAVHCOL1

VHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCACAGATCCAGTTGGTGCA 3'

VHC2 5'GTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCGCTAAGGCTTC
TGGGTATACCTTCACAAACTAG 3'

VHC3 5'GAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT
AAACACCTACACTGGAGAGCCAACA 3'

VHC4 5'TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACT
GCCTATTTGCAGATCAACACCT 3'

VHC5 5'CAAAAATGAGGACACGGCTACATATTTCGCTGCAAGAGCCTACTATGGTAAATAC
TTTGACTACGAATTC 3'

VHC6 5'GAATTCGTAGTCAAAGTATTTACCATAGTAGGCTCTTGCAGCAAATATG 3'

VHC7 5'TAGCCGTGTCCTCATTTTTGAGGTTGTTGATCTGCAAATAGGCAGTGCTGGCAGA
GGTTTCCAAAGAGAAGGCAAACCGT 3'

VHC8 5'CCCTTGAAGTCATCAGCATATGTTGGCTCTCCAGTGTAGGTGTTTATCCAGCCCAT
CCACTTTAAACCCTTTCCTGGAGC 3,

VHC9 5'CTGCTTCACCCAGTTCATTCCATAGTTTGTGAAGGTATACCCAGAAGCCTTAGCGG
AGATCTTGACTGTCTCTCCAGGCT 3'

VHC10 5'TCTTCAGCTCAGGTCCAGACTGCACCAACTGGATCTGTGCTTGGGCACTTTG GGC
AGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

FIG. 9A

2CAVLCOL1

VLC1 5'AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTT
ACCATA 3'

VLC2 5'ACCTGCAAGGCCAGTCAGAGTGTGAGTAATGATGTAGCTTGGTACCAACAGAAAACC
AGGGCAG 3'

VLC3 5'TCTCCTAAACTGCTGATATACTATGCATCCAATCGCTACACTGGAGTCCCTGATCGCT
TCACTGGCAGT 3'

VLC4 5'GGATATGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGGCA
GTTTAT 3'

VLC5 5'TTCTGYCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAG
CTGAAAGAATTC 3'

VLC6 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAATC
CTGCTGACAGAAATAAACTGC 3'

VLC7 5'CAGGTCTTCAGCCTGCACAGTGCTGATGGTGAAAGTGAAATCCGTCCCATATCCA
CTGCCAGT 3'

VLC8 5'GAAGCGATCAGGGACTCCAGTGTAGCGATTGGATGCATAGTATATCAGCAGTTTAG
GAGACTGCCCTGG 3'

VLC9 5'TTTCTGTTGGTACCAAGCTACATCATTACTCACACTCTGACTGGCCTTGCAGGTTA
TGGTAAC 3'

VLC10 5'CCTGTCTCCTGCTGATACAAGCAGGAATTTGGGAGTCTGGGTCATCACAATACTT
GCTTGGGC 3'

VLC11 5'TTCGCTCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAAGAATC 3'

VLC12 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAA
TCCTGCTGAGCGAAATAAACTGC 3'

FIG. 9B

ConVL1

Leader Sequence

L1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCA 3'

L2 5'ACTTTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKLC1 5'GATATCCAAATGACACAAAGTCCTAGTAGTTTGAGTGCTAGTGTGGGAGATCG
GGTGATCACA 3'

BKLC2 5' TGTCGGGCTAGTCAAAGTATCAGTAACTATTTGGCTTGGTATCAACAAAAGCCT
GGAAAGGCTCCTAAGTTGTTGATC 3'

BKLC3 5' TATGCTGCTAGTAGTTTGGAGAGTGGAGTGCCTAGTCGGTTCAGTGGA 3'

BKLC4 5' AGTGGAAGTGGAACACGGTTCACCTTGACCATCAGTAGTTTGCAACCTGAGGA
TTCGCTACCTATTAT 3'

BKLC5 5' TGTCAACAATATAACAGTTTGCCTTGGACCTTCGGACAAGGAACCAAGGTGGA
GATCAAGGAATTC3'

BKLC6 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAAGGTCCAAGGCAAACTGTTA
TATTGTTGACAATAATAGGT3'

BKLC7 5'AGCGAAATCCTCAGGTTGCAAACTACTGATGGTCAAGGTGAACCGTGTTCCACTT
CCACTTCCACTGAA3'

BKLC8 5'CCGACTAGGCACTCCACTCTCCAAACTACTAGCAGCATAGATCAACAA 3'

BKLC9 5' CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAAATAGTTACTGATACT
TTGACTAGCCCGACATGTGATTGT 3'

BKLC10 5'CACCCGATCTCCCACACTAGCACTCAAACTACTAGGACTTTGTGTCATTTGGA
TATCTTGCTTGGGC3'

BKLCDR12 5'TGTCGGCCTCCTGGCTTCTCTCCTTTCAGGTTGGCTTGGTATCAACAAAAGC
CTGGAAAGGCTCCTAAGTTGTTGATC 3'

BKLCDR19 5'CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAACCTGAAAGGAGA
GAAGCCAGGAGGCCGACATGTGATTGT3'

BKLCDR23 5'TATCCTGGCTTCTCTCCTTTCAGGGGAGTGCCTAGTCGGTCAGTGGA 3'

BKLCDR28 5'CCGACTAGGCACTCCCCTGAAAGGAGAGAAGCCAGGATAGATCAACAA 3'

BKLCDR35 5'TGTAGGCCTCCTGGCTTCTCTCCTTTCAGGTTCGGACAAGGAACCAAGGTGG
AGATCAAG 3'

BKLCDR36 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAACCTGAAAGGAGAGAA
GCCAGGAGGCCTACAATAATAGGT 3'

FIG. 9C

ConVH1

BKHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTG
CCCAAGCACAGATCCAGTTGGTGCAGTCTG 3'

BKHC2 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGCTTCT
GGCTACATTCACATCTTACGCTATATCTTG 3'

BKHC3 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATAAAT
GGAAATGGAGATACAATTACGCCCAGAAG 3'

BKHC4 5'TTCCAGGGAAGGGTTACTATAACTGCTGATACTTCTACTTCTACTGCTTACATGG
AGCTGTCTTCTCTGAGGTCTGAGGATACT 3'

BKHC5 5'GCTGTTTACTACTGCGCTAGGGCTCCTGGCTACGGCTCTGATTATTGGGGACA
GGGAACACTGGTTACAGTTTCTTTCTGAATTC 3'

BKHC6 5'GAATTCAGAAGAAACTGTAACCAGTGTTCCCTGTCCCCAATAATCAGAGCCGTA
GCCAGGAGCC 3'

BKHC7 5' CTAGCGCAGTAGTAAACAGCAGTATCCTCAGACCTCAGAGAAGACAGCTCCAT
GTAAGCAGTAGAAGTAGAAGTATCAGCAGTT 3'

BKHC8 5'ATAGTAACCCTTCCCTGGAACTTCTGGGCGTAATTTGTATCTCCATTTCCATTT
ATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

BKHC9 5'GAGCCTGCCTCACCCAATTCCAAGATATAGCGTAAGATGTGAATGTGTAGCCA
GAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHC10 5'AGGCTTCTTCACCTCAGCGCCAGACTGCACCAGCTGAACCTGTGCTTGGGCACT
TTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKHCDR42 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGC
TTCTGGCTACACATTCACA 3'

BKHDR43 5'CAGGTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGAT
AAATGGAGATACAAATTACGCCCAGAAG 3'

BKHDR49 5'GAGCCTGCCTCACCCACCTGAAAGGAGAGAAGCCAGGTGTGAATGTGTA
GCCAGAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHDR53 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATA
AATGGAAGGCCTCCTGGCTTCTCTCCTTTCAGG 3'

BKHDR58 5'ATAGTAACCCTTCCCTGGAACCTGAAAGGAGAGAAGCCAGGAGGCCTTC
CATTTATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

FIG. 9D

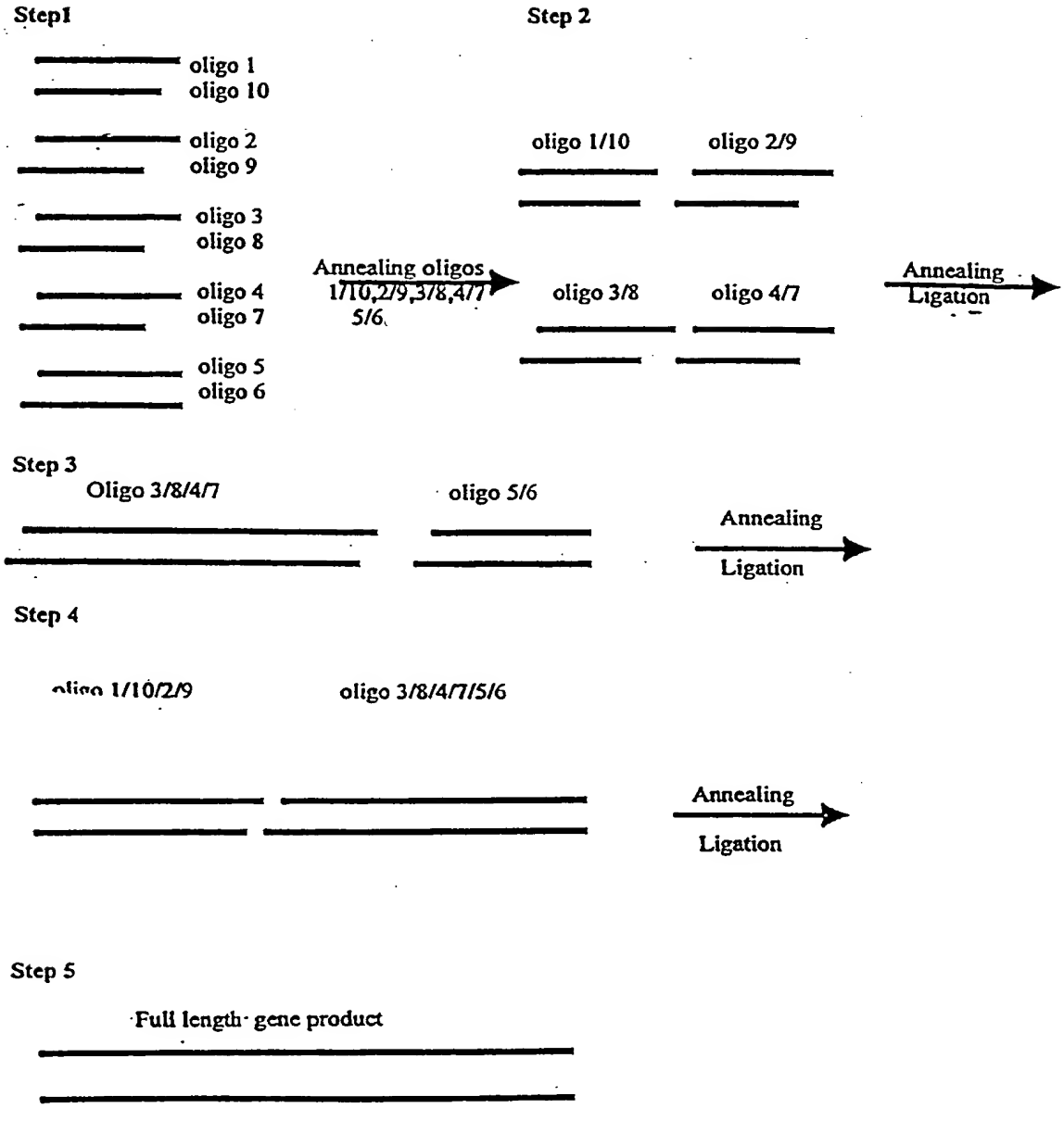

Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos 1/10,2/9,3/8,4/7 5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG. 10

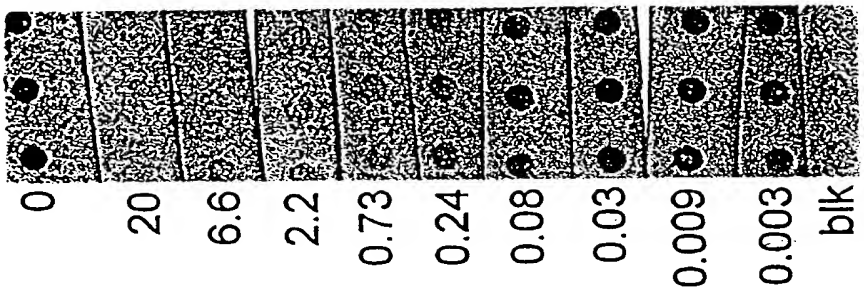
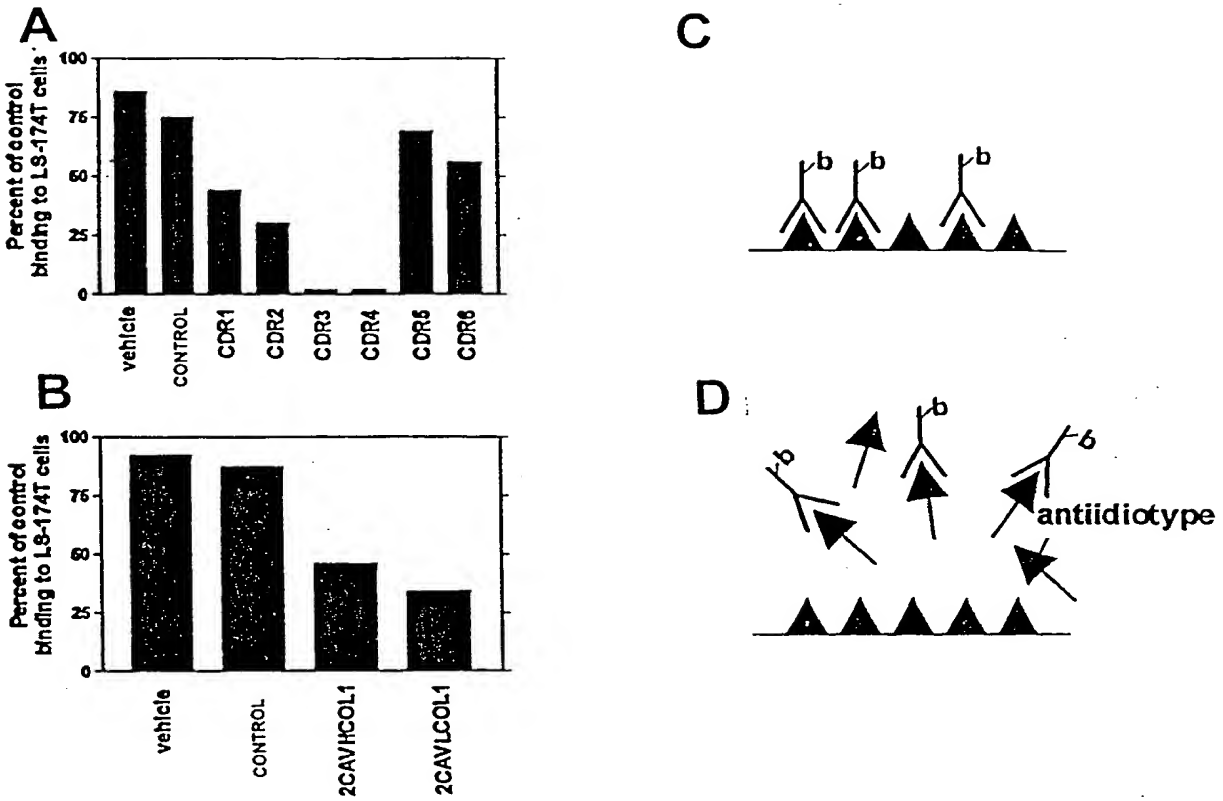


FIG. 11


A
Percent of control binding to L8-174T cells
100
75
50
25
0
vehicle
CONTROL
CDR1
CDR2
CDR3
CDR4
CDR5
CDR6
B
Percent of control binding to L8-174T cells
100
75
50
25
0
vehicle
CONTROL
2CAVHCOL1
2CAVLCOL1
C
b
b
b
D
b
b
b
antiidiotype

FIGS. 12A-D


oligo 1
1 GACATTGTGA TGTCACAGTC TCCATCCTCC CTAGCTGTGT CAGTTGGAGA
oligo 2
51 GAAGGTTACT ATGAGCTGCA AGTCCAGTCA GAGCCTTTTA TATAGTAGCA
oligo 8
101 ATCAAAAGAT CTACTTGGCC TGGTACCAGC AGAAACCAGG GCAGTCTCCT
oligo 3
151 AAACTGCTGA TTTACTGGGC ATCCACTAGG GAATCTGGGG TCCCTGATCG
oligo 7
oligo 4
201 CTTCACAGGC GGTGGATCTG GGACAGATTT CACTCTCACC ATCAGCAGTG
251 TGAAGGCTGA AGACCTGGCA GTTTATTACT GTCAGCAATA TTATAGATAT
oligo 6
301 CCTCGGACGT TCGGTGGAGG CACCAAGCTG GAAATCAAAC GG
oligo 5

FIG. 13